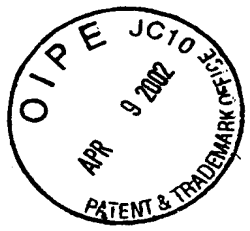

Patent Office
Canberra

I, JONNE YABSLEY, TEAM LEADER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. PR 1381 for a patent by AGRICULTURE VICTORIA SERVICES PTY. LTD. , PIG RESEARCH AND DEVELOPMENT CORPORATION and PFIZER PRODUCTS, INC. filed on 10 November 2000.

I further certify that the above application is now proceeding in the name of AGRICULTURE VICTORIA SERVICES PTY. LTD. , AUSTRALIAN PORK LIMITED and PFIZER PRODUCTS, INC. pursuant to the provisions of Section 113 of the Patents Act 1990.

WITNESS my hand this
Twenty-sixth day of November 2001

J R Yabsley

JONNE YABSLEY
TEAM LEADER EXAMINATION
SUPPORT AND SALES

Regulation 3.2

Agriculture Victoria Services Pty. Ltd.
Australian Pork Limited AND
~~Pig Research and Development Corporation~~
AND
Pfizer Products, Inc.

A U S T R A L I A

Patents Act 1990

PROVISIONAL SPECIFICATION

for the invention entitled:

"Novel therapeutic compositions for treating infection by *Lawsonia spp.*"

The invention is described in the following statement:

Novel therapeutic compositions for treating infection by *Lawsonia spp.*

FIELD OF THE INVENTION

The present invention relates generally to therapeutic compositions for the treatment and/or prophylaxis of intestinal disease conditions in animals and birds caused or exacerbated by *Lawsonia intracellularis* or similar or otherwise related microorganism. In particular, the present invention provides a novel gene derived from *L. intracellularis* which encodes an immunogenic polypeptide. The polypeptide described herein, selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides, or a homologue, analogue or derivative of any one or more of said polypeptides, is particularly useful as an antigen in vaccine preparation for conferring humoral immunity against *L. intracellularis* and related pathogens in animal hosts. The present invention is also directed to methods for the treatment and/or prophylaxis of such intestinal disease conditions and to diagnostic agents and procedures for detecting *L. intracellularis* or similar or otherwise related microorganisms.

GENERAL

Bibliographic details of the publications numerically referred to in this specification are collected at the end of the description. All patents, patent applications, and publications cited herein are incorporated by reference in their entirety.

Reference hereinafter to *"Lawsonia intracellularis"* or its abbreviation *"L. intracellularis"* includes all microorganisms similar to or otherwise related to this microorganism, as described by Stills (1991) or Jones *et al.*(1997) or Lawson *et al.* (1993) or McOrist *et al.* (1995).

References herein to "AGAL" shall be taken to mean a reference to the Australian Government Analytical Laboratories located at 1 Suakin Street, Pymble, New South Wales 2073, Australia. All biological deposits referred to herein in respect of the plasmids assigned AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR);

NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM) have been made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure.

As used herein, the word "*flhB*", or the term "*flhB* gene", shall be taken to refer to a gene encoding the antigenic flhB polypeptide of the present invention, which gene comprises the nucleotide sequence set forth in SEQ ID NO: 1 or the nucleotide sequence of the *L. intracellularis* gene contained in the plasmid pGTE#2 which has been deposited under AGAL Accession No. NM00/16477. The word *"flhB"* or the term "*flhB* gene" shall further be taken to include a degenerate or complementary nucleotide sequence to SEQ ID NO: 1 or the nucleotide sequence of the *L. intracellularis* gene contained in the plasmidpGTE#2 which has been deposited under AGAL Accession No. NM00/16477. It shall also be understood that the term "flhB polypeptide" refers to a polypeptide of the invention which comprises the amino acid sequence set forth in SEQ ID NO: 2 or a polypeptide encoded by the *L. intracellularis* gene contained in the plasmid pGTE#2 which has been deposited under AGAL Accession No. NM00/16477. The term "flhB polypeptide" shall further be taken to include a polypeptide which is functionally-related to or immunologically cross-reactive with the polypeptide of SEQ ID NO: 2 or a polypeptide encoded by the *L. intracellularis* gene contained in the plasmid pGTE#2 which has been deposited under AGAL Accession No. NM00/16477.

As used herein, the word "*fliR*", or the term "*fliR* gene", shall be taken to refer to a gene encoding the antigenic fliR polypeptide of the present invention, which gene comprises the nucleotide sequence set forth in SEQ ID NO: 3 or the nucleotide sequence of the *L. intracellularis* gene contained in the plasmid pGTE#3 which has been deposited under AGAL Accession No.NM00/16478. The word *"fliR"* or the term "*fliR* gene" shall further be taken to include a degenerate or complementary nucleotide sequence to SEQ ID NO: 3, or the nucleotide sequence of the *L. intracellularis* gene contained in the plasmid pGTE#3 which has been deposited under AGAL Accession

No.NM00/16478. It shall also be understood that the term "fliR polypeptide" refers to a polypeptide of the invention which comprises the amino acid sequence set forth in SEQ ID NO: 4 or a polypeptide encoded by the *L. intracellularis* gene contained in the plasmid pGTE#3 which has been deposited under AGAL Accession No.NM00/16478. The term "fliR polypeptide" shall further be taken to include a polypeptide which is functionally-related to or immunologically cross-reactive with the polypeptide of SEQ ID NO: 4 or a polypeptide encoded by the *L. intracellularis* gene contained in the plasmid pGTE#3 which has been deposited under AGAL Accession No.NM00/16478.

As used herein, the word "*ntrC*", or the term "*ntrC* gene", shall be taken to refer to a gene encoding the antigenic ntrC polypeptide of the present invention, which gene comprises the nucleotide sequence set forth in SEQ ID NO: 5 or the nucleotide sequence of the *L. intracellularis* gene contained in the plasmid pGTE#6 which has been deposited under AGAL Accession No.NM00/16481. The word "*ntrC*" or the term "*ntrC* gene" shall further be taken to include a degenerate or complementary nucleotide sequence to SEQ ID NO: 5, or the nucleotide sequence of the *L. intracellularis* gene contained in the plasmid pGTE#6 which has been deposited under AGAL Accession No.NM00/16481. It shall also be understood that the term "ntrC polypeptide" refers to a polypeptide of the invention which comprises the amino acid sequence set forth in SEQ ID NO: 6 or a polypeptide encoded by the *L. intracellularis* gene contained in the plasmid pGTE#6 which has been deposited under AGAL Accession No.NM00/16481. The term "ntrC polypeptide" shall further be taken to include a polypeptide which is functionally-related to or immunologically cross-reactive with the polypeptide of SEQ ID NO: 6 or a polypeptide encoded by the *L. intracellularis* gene contained in the plasmid pGTE#6 which has been deposited under AGAL Accession No.NM00/16481.

As used herein, the word "*glnH*", or the term "*glnH* gene", shall be taken to refer to a gene encoding the antigenic glnH polypeptide of the present invention, which gene comprises the nucleotide sequence set forth in SEQ ID NO: 7 or the nucleotide sequence of the *L. intracellularis* gene contained in the plasmid pGTE#1 which has

been deposited under AGAL Accession No.NM00/16476. The word *"glnH"* or the term "*glnH* gene" shall further be taken to include a degenerate or complementary nucleotide sequence to SEQ ID NO: 7, or the nucleotide sequence of the *L. intracellularis* gene contained in the plasmid pGTE#1 which has been deposited under AGAL Accession No.NM00/16476. It shall also be understood that the term "glnH polypeptide" refers to a polypeptide of the invention which comprises the amino acid sequence set forth in SEQ ID NO: 8 or a polypeptide encoded by the *L. intracellularis* gene contained in the plasmid pGTE#1 which has been deposited under AGAL Accession No.NM00/16476. The term "glnH polypeptide" shall further be taken to include a polypeptide which is functionally-related to or immunologically cross-reactive with the polypeptide of SEQ ID NO: 8 or a polypeptide encoded by the *L. intracellularis* gene contained in the plasmid pGTE#1 which has been deposited under AGAL Accession No.NM00/16476.

As used herein, the word "*motA*", or the term "*motA* gene", shall be taken to refer to a gene encoding the antigenic motA polypeptide of the present invention, which gene comprises the nucleotide sequence set forth in SEQ ID NO: 9, or to the nucleotide sequence of the *L. intracellularis* gene contained in the plasmid pGTE#4 which has been deposited under AGAL Accession No.NM00/16479 and which has homology to SEQ ID NO: 9. The word *"motA"* or the term "*motA* gene" shall further be taken to include a degenerate or complementary nucleotide sequence to SEQ ID NO: 9, or the nucleotide sequence of the *L. intracellularis* gene contained in the plasmid pGTE#4 which has been deposited under AGAL Accession No.NM00/16479 and which has homology to SEQ ID NO: 9. It shall also be understood that the term "motA polypeptide" refers to a polypeptide of the invention which comprises the amino acid sequence set forth in SEQ ID NO: 10 or a polypeptide encoded by the *L. intracellularis* gene contained in the plasmid pGTE#4 which has been deposited under AGAL Accession No.NM00/16479 and which has homology to SEQ ID NO: 9. The term "motA polypeptide" shall further be taken to include a polypeptide which is functionally-related to or immunologically cross-reactive with the polypeptide of SEQ ID NO: 10 or a polypeptide encoded by the *L. intracellularis* gene contained in the plasmid pGTE#4

which has been deposited under AGAL Accession No.NM00/16479 and having homology to SEQ ID NO: 9.

As used herein, the word "*motB*", or the term "*motB* gene", shall be taken to refer to a gene encoding the antigenic motB polypeptide of the present invention, which gene comprises the nucleotide sequence set forth in SEQ ID NO: 11 or the nucleotide sequence of the *L. intracellularis* gene contained in the plasmid pGTE#4 which has been deposited under AGAL Accession No.NM00/16479 and having homology to SEQ ID NO: 11. The word "*motB*" or the term "*motB* gene" shall further be taken to include a degenerate or complementary nucleotide sequence to SEQ ID NO: 11, or the nucleotide sequence of the *L. intracellularis* gene contained in the plasmid pGTE#4 which has been deposited under AGAL Accession No.NM00/16479 and having homology to SEQ ID NO: 11. It shall also be understood that the term "motB polypeptide" refers to a polypeptide of the invention which comprises the amino acid sequence set forth in SEQ ID NO: 12 or a polypeptide encoded by the *L. intracellularis* gene contained in the plasmid pGTE#4 which has been deposited under AGAL Accession No.NM00/16479 and having homology to SEQ ID NO: 11. The term "motB polypeptide" shall further be taken to include a polypeptide which is functionally-related to or immunologically cross-reactive with the polypeptide of SEQ ID NO: 12 or a polypeptide encoded by the *L. intracellularis* gene contained in the plasmid pGTE#4 which has been deposited under AGAL Accession No.NM00/16479 and having homology to SEQ ID NO: 11.

As used herein, the word "*tlyC*", or the term "*tlyC* gene", shall be taken to refer to a gene encoding the antigenic tlyC polypeptide of the present invention, which gene comprises the nucleotide sequence set forth in SEQ ID NO: 13 or the nucleotide sequence of the *L. intracellularis* gene contained in the plasmid pGTE#5 which has been deposited under AGAL Accession No.NM00/16480. The word "*tlyC*" or the term "*tlyC* gene" shall further be taken to include a degenerate or complementary nucleotide sequence to SEQ ID NO: 13, or the nucleotide sequence of the *L. intracellularis* gene contained in the plasmid pGTE#5 which has been deposited under AGAL Accession

No.NM00/16480. It shall also be understood that the term "tlyC polypeptide" refers to a polypeptide of the invention which comprises the amino acid sequence set forth in SEQ ID NO: 14 or a polypeptide encoded by the *L. intracellularis* gene contained in the plasmid pGTE#5 which has been deposited under AGAL Accession No.NM00/16480. The term "tlyC polypeptide" shall further be taken to include a polypeptide which is functionally-related to or immunologically cross-reactive with the polypeptide of SEQ ID NO: 14 or a polypeptide encoded by the *L. intracellularis* gene contained in the plasmid pGTE#5 which has been deposited under AGAL Accession No.NM00/16480.

As used herein, the word "*ytfM*", or the term "*ytfM* gene", shall be taken to refer to a gene encoding the antigenic ytfM polypeptide of the present invention, which gene comprises the nucleotide sequence set forth in SEQ ID NO: 15 or the nucleotide sequence of the *L. intracellularis* gene contained in the plasmid pGTE#7 which has been deposited under AGAL Accession No.NM00/16482. The word *"ytfM"* or the term "*ytfM* gene" shall further be taken to include a degenerate or complementary nucleotide sequence to SEQ ID NO: 15, or the nucleotide sequence of the *L. intracellularis* gene contained in the plasmid pGTE#7 which has been deposited under AGAL Accession No.NM00/16482. It shall also be understood that the term "ytfM polypeptide" refers to a polypeptide of the invention which comprises the amino acid sequence set forth in SEQ ID NO: 16 or a polypeptide encoded by the *L. intracellularis* gene contained in the plasmid pGTE#7 which has been deposited under AGAL Accession No.NM00/16482.
The term "ytfM polypeptide" shall further be taken to include a polypeptide which is functionally-related to or immunologically cross-reactive with the polypeptide of SEQ ID NO: 16 or a polypeptide encoded by the *L. intracellularis* gene contained in the plasmid pGTE#7 which has been deposited under AGAL Accession No.NM00/16482.

As used herein, the word "*ytfN*", or the term "*ytfN* gene", shall be taken to refer to a gene encoding the antigenic ytfN polypeptide of the present invention, which gene comprises the nucleotide sequence set forth in SEQ ID NO: 17. The word *"ytfN"* or the term "*ytfN* gene" shall further be taken to include a degenerate or complementary

nucleotide sequence to SEQ ID NO: 17. It shall also be understood that the term "ytfN polypeptide" refers to a polypeptide of the invention which comprises the amino acid sequence set forth in SEQ ID NO: 18. The term "ytfN polypeptide" shall further be taken to include a polypeptide which is functionally-related to or immunologically cross-reactive with the polypeptide of SEQ ID NO: 18.

As used herein the words "from" or "of", and the term "derived from" shall be taken to indicate that a specified product, in particular a macromolecule such as a polypeptide, protein, gene or nucleic acid molecule, antibody molecule, Ig fraction, or other macromolecule, or a biological sample comprising said macromolecule, may be obtained from a particular source, organism, tissue, organ or cell, albeit not necessarily directly from that source, organism, tissue, organ or cell.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated step or element or integer or group of steps or elements or integers but not the exclusion of any other step or element or integer or group of elements or integers.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations or any two or more of said steps, features, compositions and compounds.

The present invention is not to be limited in scope by the specific embodiments described herein, which are intended for the purposes of exemplification only. Functionally equivalent products, compositions and methods are clearly within the scope of the invention, as described herein.

BACKGROUND OF THE INVENTION

The meat-producing sector of the agricultural industry is dependent upon the health of its livestock and there is a need to maintain disease-free livestock for human consumption. The industry is subject to rapid economic downturn in response to disease conditions adversely affecting livestock and the quality of meat products derived therefrom, including those diseases which may potentially be transmitted to humans. It is important, therefore, to have well defined treatments and prophylactic and diagnostic procedures available to deal with infections or potential infections in livestock animals and humans.

Meat products derived from porcine and avian species are significant commercial products in the agriculture industry. In particular, pigs form a major component of the meat industry. However, pigs are sensitive to a wide spectrum of intestinal diseases collectively referred to as porcine proliferative enteropathy (PPE). These diseases have previously been known as intestinal adenomatosis complex (Barker and van Drumel, 1985), porcine intestinal adenomatosis (PIA), necrotic enteritis (Rowland and Lawson, 1976), proliferative haemorrhagic enteropathy (Love and Love, 1977), regional ileitis (Jonsson and Martinsson, 1976), haemorrhagic bowel syndrome (O'Neil, 1970), porcine proliferative enteritis and *Campylobacter* spp – induced enteritis (Straw, 1990).

There are two main forms of PPE: a non-haemorrhagic form represented by intestinal adenomatosis which frequently causes growth retardation and mild diarrhoea; and a haemorrhagic form, which is often fatal, represented by proliferative haemorrhagic enteropathy (PHE), where the distal small intestine lumen becomes engorged with blood. PPE has been reported in a number of animal species including pigs (McOrist *et al*, 1993), hamsters (Stills, 1991), ferrets (Fox *et al*, 1989), guinea pigs (Elwell *et al*, 1981), rabbits (Schodeb and Fox, 1990) as well as avian species (Mason *et al*, 1998).

PPE is a significant cost component associated with the pig industry, especially in terms of stock losses, medication costs, reduced growth rates of pigs and increased

feed costs. PPE also contributes to downstream indirect costs in, for example, additional labour costs and environmental costs in dealing with antibiotic residue contamination, and in control measures to prevent the organism from being passed on or carried to other animals or humans.

L. intracellularis is a causative agent of PPE (McOrist *et al*, 1995). *L. intracellularis* is an intracellular, possibly obligate intracellular, bacterium. It can only be cultured *in vitro* with tissue culture cells (Jones *et al.*, 1997; Lawson *et al.*, 1993; McOrist *et al*, 1995; International Patent Application No. PCT/US96/09576). *L. intracellularis* is located in the cytoplasm of the villus cells and intestinal crypt cells of infected animals. Pigs suffering from PPE are characterised by irregularities in the villus cells and intestinal crypt structure with epithelial cell dysplasia, wherein crypt abscesses form as the villi and intestinal crypts become branched and fill with inflammatory cells.

Current control strategies for PPE rely on the use of antibacterials. However, such a strategy is considered to only be short to medium term, especially since governmental regulatory pressures tend to discourage animal husbandry practices which involve the use of prophylactic antibiotics. There is a need, therefore, to develop effective, safe and low cost alternatives to the use of antibiotics and, in particular, to develop vaccine preparations capable of conferring protective immunity against *L. intracellularis* infection in livestock animals.

The most effective vaccine preparations are generally comprised of a highly antigenic component, such as a polypeptide or other macromolecule which is derived from the pathogenic organism against which the vaccine is directed, wherein said antigenic component produces little or no contraindications when administered to a susceptible host animal, and produces little or no antigenic cross-reactivity with desirable organisms, such as non-pathogenic organisms that are a part of the normal flora of the intestinal tract or other tissues of said host animal. In summary, an effective vaccine preparation must be immunogenic, specific and safe.

Accordingly, there is a need to identify highly immunogenic antigens produced by the bacterium *L. intracellularis*.

International Patent Application No. PCT/AU96/00767 describes several *L. intracellularis* partial genetic sequences, and partial polypeptides encoded thereby. However, there is a need to further identify polypeptide immunogens produced by the bacterium *L. intracellularis* and immunogenic peptides derived therefrom, including those immunogens which are genus- or species-specific, for use in improved vaccine compositions. The presently-described invention provides such immunogens.

SUMMARY OF THE INVENTION

One aspect of the present invention is directed to an isolated or recombinant immunogenic polypeptide which comprises, mimics or cross-reacts with a B-cell or T-cell epitope of a polypeptide derived from *Lawsonia spp,* in particular a polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides, or a homologue, analogue or derivative of any one or more of said polypeptides.

Preferably, the isolated or recombinant immunogenic polypeptide is selected from the group consisting of the following:

(i) a polypeptide which comprises an amino acid sequence which has at least about 60% sequence identity overall to an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, and 18;

(ii) a polypeptide which comprises an amino acid sequence which has at least about 60% sequence identity overall to an amino acid sequence encoded by *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM);

(iii) a polypeptide which comprises at least about 5 contiguous amino acids of an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, and 18;

(iv) a polypeptide which comprises at least about 5 contiguous amino acids of an amino acid sequence encoded by *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM); and

(v) a homologue, analogue or derivative of any one of (i) to (iv) which mimics a B-cell or T-cell epitope of *Lawsonia spp.*

In an alternative preferred embodiment, the isolated or recombinant immunogenic polypeptide is selected from the group consisting of the following:

(i) a polypeptide which comprises an amino acid sequence encoded by a nucleotide sequence having at least about 60% sequence identity overall to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17;

(ii) a polypeptide which comprises an amino acid sequence encoded by a nucleotide sequence having at least about 60% sequence identity overall to the nucleotide sequence of *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM);

(iii) a polypeptide encoded by at least about 15 contiguous nucleotides of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17;

(iv) a polypeptide encoded by at least about 15 contiguous nucleotides of a

nucleotide sequence of *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM); and

(v) a homologue, analogue or derivative of any one of (i) to (iv) which mimics a B-cell or T-cell epitope of *Lawsonia spp.*

In a particularly preferred embodiment, the polypeptide of the present invention comprises or consists of an amino acid sequence selected from the group consisting of:

(i) an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, and 18; and

(ii) an amino acid sequence encoded by *L. intracellularis* DNA contained within a deposited plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM).

A further aspect of the present invention provides a vaccine composition for the prophylaxis or treatment of infection in an animal, such as a pig or bird, by *L. intracellularis* or a similar or otherwise related microorganism, said vaccine composition comprising an immunologically effective amount of an immunogenic component which comprises an isolated or recombinant polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides as described herein and one or more carriers, diluents and/or adjuvants suitable for veterinary or pharmaceutical use.

A further aspect of the invention extends to an immunologically interactive molecule,

such as an antibody or antibody fragment, which is capable of binding to an immunogenic polypeptide of the invention selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides.

A further aspect of the invention provides a method of diagnosing infection of an animal by *L. intracellularis* or a related microorganism, said method comprising the steps of contacting a biological sample derived from said animal with an immunologically interactive molecule of the present invention for a time and under conditions sufficient for a complex, such as an antigen:antibody complex, to form, and then detecting said complex formation.

A further aspect of the present invention contemplates a method of determining whether or not an animal has suffered from a past infection, or is currently infected, by *L. intracellularis* or a related microorganism, said method comprising contacting a tissue or fluid sample, such as blood or serum derived from said animal, with an immunogenic polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides, or a peptide derived therefrom, for a time and under conditions sufficient for a complex, such as an antigen:antibody complex, to form, and then detecting said complex formation.

A further aspect of the present invention provides an isolated nucleic acid molecule which comprises a sequence of nucleotides that encodes, or is complementary to a nucleic acid molecule that encodes, a polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides, including any and all genes selected from the group consisting of *flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM*, and *ytfN* genes as defined hereinabove.

In a preferred embodiment, the isolated nucleic acid molecule comprises a nucleotide sequence encoding a polypeptide that is immunologically cross-reactive with *L. intracellularis* or other causative agent of PPE, wherein said nucleotide sequence is selected from the group consisting of:

(i) a nucleotide sequence having at least about 60% sequence identity overall to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17;

(ii) a nucleotide sequence having at least about 60% sequence identity overall to *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM);

(iii) a nucleotide sequence which comprises at least about 15 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17;

(iv) a nucleotide sequence which comprises at least about 15 contiguous nucleotides of *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM);

(v) a nucleotide sequence which hybridizes under at least low stringency, more preferably moderate stringency, and most preferably high stringency conditions to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17 or a complementary nucleotide sequence thereto;

(vi) a nucleotide sequence which hybridizes under at least low stringency, more preferably moderate stringency, and most preferably high stringency conditions to *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5

tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM); and

(vii) a homologue, analogue or derivative of any one of (i) to (vi) which encodes a polypeptide which mimics a B-cell or T-cell epitope of *Lawsonia spp.*

In a particularly preferred embodiment, the isolated nucleic acid molecule of the present invention comprises or consists of a nucleotide sequence selected from the group consisting of:

(i) a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1,3, 5, 7, 9, 11, 13, 15, and 17;

(ii) a nucleotide sequence of the *L. intracellularis* DNA contained within a deposited plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM); and

(iii) a nucleotide sequence that encodes the same polypeptide as (i) or (ii), wherein said polypeptide is selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN;

(iv) a nucleotide sequence that is complementary to (i) or (ii) or (iii); and

(v) a nucleotide sequence that hybridises under high stringency conditions to the complement of a sequence selected from the group consisting of: SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15 and 17, wherein said nucleotide sequence is the complement of a sequence that encodes a polypeptide that is immunologically cross-reactive to a polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN.

A still further aspect of the invention provides a diagnostic method of detecting *L. intracellularis* or related microorganism in a biological sample derived from an animal subject, said method comprising the steps of hybridising one or more polynucleotide or oligonucleotide probes or primers derived from a gene selected from the group

consisting of *flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM*, and *ytfN* genes, or a homologue, analogue or derivative thereof, to said sample, and then detecting said hybridisation using a detection means. The detection means according to this aspect of the invention is any nucleic acid-based hybridisation or amplification reaction.

A further aspect of the invention provides an isolated probe or primer derived from a gene selected from the group consisting of *flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM*, and *ytfN* genes. In a particularly preferred embodiment, the probe or primer of the invention is useful for isolating the *ytfM* and/or *ytfN* genes described herein. More preferably, the probe or primer of the invention comprises a nucleotide sequence selected from the group consisting of SEQ ID NO: 19 to SEQ ID NO: 46 or a complementary nucleotide sequence thereto.

DETAILED DESCRIPTION OF THE INVENTION

In work leading up to the present invention, the inventors sought to identify immunogenic proteins of *L. intracellularis* for use in vaccines for the prophylaxis and treatment of PPE in animals, including pigs and birds.

Accordingly, one aspect of the present invention is directed to an isolated or recombinant immunogenic polypeptide which comprises, mimics or cross-reacts with a B-cell or T-cell epitope of a polypeptide derived from *Lawsonia spp,* selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN, or a homologue, analogue or derivative of any one or more of said polypeptides.

Epitopes of *Lawsonia spp.* may be B cell epitopes or T-cell epitopes. It is well-known that antibody-binding sites (B-cell epitopes) involve linear as well as conformational epitopes (van Regenmortel, 1992). B-cell epitopes are predominantly conformational. In contrast, T-cells recognize predominantly linear epitope sequences in combination with MHC class II molecules.

A precise identification and careful selection of epitopes of *Lawsonia spp.* facilitates

the development of diagnostic reagents and vaccine compositions for the effective treatment or prophylaxis of *Lawsonia* infections. Epitope identification and characterization (i.e., determination of the molecular weight, amino acid sequence, and structure of epitopes of *Lawsonia spp.*) may be performed using art-recognised techniques. For the detection of conformational epitopes, degrading and denaturing of the epitope molecule must be avoided in order to conserve the three-dimensional structure, because the antigen-antibody reaction will be diminished if the secondary structure of the epitope is altered significantly. In practice, the characterisation and isolation of linear non-conformational epitopes is easier, because any immunoreactive regions are contained within a single polypeptide or peptide fragment which is capable of being purified under a range of conditions.

Both non-conformational and conformational epitopes may be identified by virtue of their ability to bind detectable amounts of antibodies (such as IgM or IgG) from sera of animals immunised against or infected with *Lawsonia spp.* and, in particular *L. intracellularis,* or an isolated polypeptide derived therefrom or, alternatively, by virtue of their ability to bind detectable amounts of antibodies in a purified Ig fraction derived from such sera. The antibodies may be derived from or contained within pools of polyclonal sera, or may be monoclonal antibodies. Antibody fragments or recombinant antibodies, such as those expressed on the surface of a bacteriophage or virus particle, such as in a phage display library, may also be employed.

The determination of T-cell epitopes is performed by analysing the ability of the epitope peptides to induce the proliferation of peripheral blood lymphocytes or T-cell clones. The identification of T-cell epitopes is accomplished using a variety of methods as known in the art, including the use of whole and fragmented native or recombinant antigenic protein, as well as the more commonly employed "overlapping peptide" method. In the latter method, overlapping peptides which span the entire sequence of a polypeptide derived from *Lawsonia spp.* are synthesized and tested for their capacity to stimulate T-cell cytotoxic or proliferative responses *in vitro*.

Structure determination of both conformational non-linear and non-conformational linear epitopes may be performed by nuclear magnetic resonance spectroscopy (NMR) and X-ray crystallographic analysis. The determination of epitopes using X-ray techniques requires the protein-antibody complex to be crystallized, whereas NMR allows analysis of the complex in a liquid state. NMR measures the amount of amino acids as well as the neighbourhood of protons of different amino acid residues, wherein the alternating effect of two protons along the carbon backbone is characteristic of a particular epitope.

A successful method to recognize non-conformational linear epitopes is the immunoblot and in particular, the Western blot. Peptides may be generated from a complete *Lawsonia spp.* polypeptide by digestion with site-specific proteases, such as trypsin or chymotrypsin, and the peptides generated thereby can be separated using standard electrophoretic or chromatographic procedures. For example, after electrophoresis according to molecular weight using SDS/PAGE (SDS/polyacrylamide gel electrophoresis) and/or according to isoelectric point using IEF (isoelectric focussing) or alternatively, by two-dimensional electrophoresis, the peptides can be transferred to immobilizing nylon or nitrocellulose membranes and incubated with sera raised against the intact polypeptides. Peptides that comprise immunogenic regions (i.e., B-cell or T-cell epitopes) are bound by the antibodies in the sera and the bound antibodies may be detected using secondary antibodies, such as anti-IgG antibodies, that have been labelled radioactively or enzymatically. The epitopes may then be characterised by purification based upon their size, charge or ability to bind specifically to antibodies against the intact polypeptide, using one or more techniques, such as size-exclusion chromatography, ion-exchange chromatography, affinity chromatography or ELISA among others. After purification of the epitope, only one band or spot should be detectable with gel electrophoresis. The N-terminal or total sequencing of the polypeptide or peptide fragment offers the possibility to compare the amino acid sequence with known proteins in databases.

Several computer-driven algorithms have now been devised to search for T-cell epitopes in proteins (Margalit *et al*, 1987; Vajda and C. DeLisi, 1990; Altuvia *et al.*,

1994; Parker *et al.*1994; DeGroot *et al.*,1995; Gabriel *et al*, 1995; Meister *et al.*, 1995). These algorithms search the amino acid sequence of a given protein for characteristics believed to be common to immunogenic peptides, locating regions that are likely to induce a cellular immune response *in vitro*. Computer-driven algorithms can identify regions of a *Lawsonia spp.* polypeptide that contain epitopes and are less variable among different isolates. Alternatively, computer-driven algorithms can rapidly identify regions of each isolate's more variable proteins that should be included in a multivalent vaccine.

The AMPHI algorithm (Margalit *et al.*,1987), which is based on the periodicity of T cell epitopes, has been widely used for the prediction of T-cell antigenic sites from sequence information alone. Essentially, AMPHI describes a common structural pattern of MHC binding motifs, since MHC binding motifs (i.e., patterns of amino acids that appear to be common to most of the peptides that bind to a specific MHC molecule) appear to exhibit the same periodicity as an alpha helix. Identification of T-cell epitopes by locating MHC binding motifs in an amino acid sequence provides an effective means of identifying immunogenic epitopes in diagnostic assays.

The EpiMer algorithm (Meister *et al.*, 1995; Gabriel *et al.*, 1995; DeGroot *et al.*, 1995) locates clustered MHC binding motifs in amino acid sequences of proteins, based upon the correlation between MHC binding motif-dense regions and peptides that may have the capacity to bind to a variety of MHC molecules (promiscuous or multi-determinant binders) and to stimulate an immune response in these various MHC contexts as well (promiscuous or multi-determinant epitopes). The EpiMer algorithm uses a library of MHC binding motifs for multiple class I and class II HLA alleles to predict antigenic sites within a protein that have the potential to induce an immune response in subjects with a variety of genetic backgrounds. EpiMer locates matches to each MHC-binding motif within the primary sequence of a given protein antigen. The relative density of these motif matches is determined along the length of the antigen, resulting in the generation of a motif-density histogram. Finally, the algorithm identifies protein regions in this histogram with a motif match density above an algorithm-defined

cutoff density value, and produces a list of subsequences representing these clustered, or motif-rich regions. The regions selected by EpiMer may be more likely to act as multi-determinant binding peptides than randomly chosen peptides from the same antigen, due to their concentration of MHC-binding motif matches. The selection of regions that are MHC binding motif-dense increases the likelihood that the predicted polypeptide or peptide fragment contains a "valid" motif, and furthermore, that the reiteration of identical motifs may contribute to binding.

Additional MHC binding motif-based algorithms have been described by Parker *et al.*(1994) and Altuvia *et al.*(1994). In these algorithms, binding to a given MHC molecule is predicted by a linear function of the residues at each position, based on empirically defined parameters, and in the case of the Altuvia *et al.*(1994) algorithm, known crystallographic structures may also be taken into consideration.

Recombinant methods offer the opportunity to obtain well characterized epitopes of high purity for the production of diagnostic reagents and epitope-specific vaccine formulations (Mohapatra *et al.*, 1995). Based upon the amino acid sequence of a linear epitope and identification of the corresponding nucleotide sequence encoding same, polymerase chain reaction (PCR) may be performed to amplify the epitope-encoding region from cDNA. After cloning and expression in a suitable vector/host system, a large amount of epitopes of high purity can be extracted. Accordingly, the present invention clearly extends to both isolated non-recombinant polypeptides and recombinant polypeptides in an impure or isolated form.

The term "polypeptide" as used herein shall be taken to refer to any polymer consisting of amino acids linked by covalent bonds and includes within its scope the full-length amino acids disclosed herein, and any parts or fragments thereof such as, for example, peptides consisting of about 5-50 amino acid residues in length, preferably about 5-30 amino acid residues in length, more preferably about 5-20 amino acid residues in length, and even more preferably about 5-10 amino acid residues in length. Also included within the scope of the definition of a "polypeptide" are amino acid

sequence variants, containing one or more preferably conservative amino acid substitutions, deletions, or insertions, which do not alter at least one essential property of said polypeptide such as, for example, its immunogenicity, use as a diagnostic reagent, or effectiveness as a vaccine against *Lawsonia spp*, amongst others. Accordingly, a polypeptide may be isolated from a source in nature, or chemically synthesized. Furthermore, a polypeptide may be derived from a full-length protein by chemical or enzymatic cleavage, using reagents such as CNBr, trypsin, or chymotrypsin, amongst others.

Conservative amino acid substitutions are well-known in the art. For example, one or more amino acid residues of a native flagellar hook protein of the present invention can be substituted conservatively with an amino acid residue of similar charge, size or polarity, with the resulting polypeptide retaining an ability to function in a vaccine or as a diagnostic reagent as described herein. Rules for making such substitutions include those described by Dayhof (1978). More specifically, conservative amino acid substitutions are those that generally take place within a family of amino acids that are related in their side chains. Genetically-encoded amino acids are generally divided into four groups: (1) acidic=aspartate, glutamate; (2) basic=lysine, arginine, and histidine; (3) non-polar=alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, and tryptophan; and (4) uncharged polar= glycine, asparagine, glutamine, cysteine, serine, threonine, and tyrosine. Phenylalanine, tyrosine and tryptophan are also jointly classified as aromatic amino acids. One or more replacements within any particular group such as, for example, the substitution of leucine for isoleucine or valine or alternatively, the substitution of aspartate for glutamate or threonine for serine, or of any other amino acid residue with a structurally-related amino acid residue, will generally have an insignificant effect on the function of the resulting polypeptide.

The present invention is not limited by the source of the subject immunogen and clearly extends to isolated and recombinant polypeptides which are derived from a natural or a non-natural occurring source.

The term "recombinant polypeptide" as used herein shall be taken to refer to a polypeptide which is produced *in vitro* or in a host cell by the expression of a genetic sequence encoding said polypeptide, which genetic sequence is under the control of a suitable promoter, wherein a genetic manipulation has been performed in order to achieve said expression. Accordingly, the term "recombinant polypeptide" clearly encompasses polypeptides produced by the expression of genetic sequences contained in viral vectors, cosmids or plasmids that have been introduced into prokaryotic or eukaryotic cells, tissues or organs. Genetic manipulations which may be used in this context will be known to those skilled in the art and include, but are not limited to, nucleic acid isolation, restriction endonuclease digestion, exonuclease digestion, end-filling using the Klenow fragment of *E. coli* DNA polymerase I or T4 DNA polymerase enzymes, blunt-ending of DNA molecules using T4 DNA polymerase or ExoIII enzymes, site-directed mutagenesis, ligation, and amplification reactions. As will be known to those skilled in the art, additional techniques such as nucleic acid hybridisations and nucleotide sequence analysis may also be utilised in the preparation of recombinant polypeptides, in confirming the identity of a nucleic acid molecule encoding a desired recombinant polypeptide and a genetic construct comprising the nucleic acid molecule.

Wherein the polypeptide of the present invention is a recombinant polypeptide, it may be produced in and, if desirable, isolated from a recombinant viral vector expression system or host cell. As will be known to those skilled in the relevant art, a cell for production of a recombinant polypeptide is selected on the basis of several parameters including the genetic constructs used to express the polypeptide under consideration, as well as the stability and activity of said polypeptide. It will also be known to those skilled in the art that the stability or activity of a recombinant polypeptide may be determined, at least in part, by post-translational modifications to the polypeptide such as, for example, glycosylation, acylation or alkylation reactions, amongst others, which may vary between cell lines used to produce the recombinant polypeptide.

Accordingly, in a more particularly preferred embodiment, the present invention extends to a recombinant polypeptide or a derivative, homologue or analogue thereof

as present in a virus particle, or as produced in prokaryotic or eukaryotic host cell, or in a virus or cell culture thereof.

The present invention also extends to a recombinant polypeptide according to any of the foregoing embodiments which is produced in a bacterial cell belonging to the genus *Lawsonia*, in particular a cell of *L. intracellularis,* or a culture thereof.

The term "isolated polypeptide" refers to a polypeptide of the present invention which has been purified to some extent, preferably to at least about 20% by weight of protein, preferably to at least about 50% by weight of protein, more preferably to at least about 60% by weight of protein, still more preferably to at least about 70% by weight of protein and even more preferably to at least about 80% by weight of protein or greater, from its natural source or, in the case of non-naturally-occurring polypeptides, from the culture medium or cellular environment in which it was produced. Such isolation may be performed to improve the immunogenicity of the polypeptide of the present invention, or to improve the specificity of the immune response against that polypeptide, or to remove toxic or undesirable contaminants therefrom. The necessary or required degree of purity of an isolated polypeptide will vary depending upon the purpose for which the polypeptide is intended, and for many applications it will be sufficient for the polypeptide preparation to contain no contaminants which would reduce the immunogenicity of the polypeptide when administered to a host animal, in particular a porcine or avian animal being immunized against PPE or, alternatively, which would inhibit immuno-specific binding in an immunoassay for the diagnosis of PPE or a causative agent thereof.

The purity of an isolated polypeptide of the present invention may be determined by any means known to those skilled in the art, including the degree of homogeneity of a protein preparation as assessed by SDS/polyacrylamide gel electrophoresis, 2-dimensional electrophoresis, or amino acid composition analysis or sequence analysis.

Preferably, the polypeptide of the present invention will be substantially homogeneous or substantially free of nonspecific proteins, as assessed by SDS/polyacrylamide gel

electrophoresis, 2-dimensional electrophoresis, or amino acid composition analysis or sequence analysis.

The polypeptide of the present invention can be purified for use as a component of a vaccine composition by any one or a combination of methods known to those of ordinary skill in the art, including, for example, reverse phase chromatography, HPLC, ion-exchange chromatography, and affinity chromatography, among others.

In a preferred embodiment, the isolated or recombinant polypeptide of the invention functions is secretable into the periplasmic space of a cell, preferably into the periplasm of a prokaryotic cell, such as, for example, *Escherichia coli.* or *L. intracellularis,* or, alternatively, is immunologically cross-reactive with a *L. intracellularis* polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN.

In a particularly preferred embodiment, the isolated or recombinant polypeptide of the invention is derived from *Lawsonia spp.* or other pathogenic agent associated with the onset and/or development of PPE and more preferably, the subject polypeptide is derived from *L. intracellularis*.

A B-cell or T-cell epitope of a polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides, or a homologue, analogue or derivative of any one or more of said polypeptides, may comprise one or more of the following:

(i) the primary amino acid sequence of any one of said polypeptides, as determined by an art-accepted methodology to comprise a continuous non-conformational epitope;

(ii) the secondary structure which any one of said polypeptides adopt, as determined by an art-accepted methodology to comprise a continuous conformational epitope;

(iii) the tertiary structure which any one of said polypeptides adopt in contact with another region of the same polypeptide molecule, as determined by an art-accepted methodology to comprise a discontinuous conformational epitope; or

(iv) the quaternary structure which any one of said polypeptides adopt in contact with a region of another polypeptide molecule, as determined by an art-accepted methodology to comprise a discontinuous conformational epitope.

Accordingly, immunogenic polypeptides or derivatives, homologues or analogues thereof comprising the same, or substantially the same primary amino acid sequence are hereinafter defined as "immunogens which comprise a B-cell or T-cell epitope" or similar term.

Immunogenic polypeptides or derivatives, homologues, or analogues thereof comprising different primary amino acid sequences may comprise immunologically identical immunogens, because they possess conformational B-cell or T-cell epitopes that are recognised by the immune system of a host species to be identical. Such immunogenic polypeptides or derivatives, homologues or analogues thereof are hereinafter defined as "immunogens which mimic or cross-react with a B-cell or T-cell epitope", or similar term.

Accordingly, the present invention extends to an immunogen which comprises, mimics, or cross-reacts with a B-cell or T-cell epitope of an isolated or recombinant polypeptide according to any one of the foregoing embodiments or a derivative, homologue or analogue thereof. In a particularly preferred embodiment, the present invention provides an immunogen which comprises, mimics, or cross-reacts with a B-cell or T-cell epitope of an isolated or recombinant polypeptide which in its native form is obtainable from a species of *Lawsonia* such as, but not limited to *L. intracellularis* and which polypeptide preferably has the same biological function as a polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN, as hereinbefore defined.

Preferably, such immunogenic polypeptides will not comprise a primary amino acid sequence which is highly-conserved between *L. intracellularis* and another non-pathogenic microorganism which is normally resident in the gut or other organ of an animal, in particular a porcine or avian animal. The significance of this exclusion to those embodiments of the invention wherein specificity is essential to performance (eg vaccine and diagnostic applications) will be apparent to those skilled in the art.

To improve the immunogenicity of a subject polypeptide of the present invention one or more amino acids not corresponding to the original protein sequence can be added to the amino or carboxyl terminus of the polypeptide. Such extra amino acids are useful for coupling the polypeptide to another peptide or polypeptide, to a large carrier protein or to a solid support. Amino acids that are useful for these purposes include but are not limited to tyrosine, lysine, glutamic acid, aspartic acid, cysteine and derivatives thereof. Additional protein modification techniques can be used such as, e.g., NH_2-acetylation or COOH-terminal amidation, to provide additional means for coupling the polypeptide to another polypeptide or peptide molecule, or to a solid support. Procedures for coupling polypeptides to each other, or to carrier proteins or solid supports, are well known in the art. Polypeptides containing the above-mentioned extra amino acid residues at either the carboxyl- or amino-termini and either uncoupled or coupled to a carrier or solid support, are consequently within the scope of the present invention.

Furthermore, the polypeptide can be immobilised to a polymeric carrier or support material.

In an alternative embodiment, the immunogenicity of a polypeptide of the present invention may be improved using molecular biology techniques to produce a fusion protein containing one or more polypeptides of the present invention fused to a carrier molecules such as a highly immunogenic protein. For example, a fusion protein containing a polypeptide of the present invention fused to the highly immunogenic B subunit of cholera toxin can be used to increase the immune response to the

polypeptide. The present invention also contemplates fusion proteins comprising a cytokine, such as an interleukin, fused to the subject polypeptide of the present invention, and genes encoding same.

Preferably, the polypeptide of the present invention, or a derivative, homologue or analogue thereof, when administered to a mammal, induces an immune response in said mammal. More preferably, the polypeptide of the present invention, when administered to a mammal, in particular a porcine animal (e.g., a pig) induces a protective immune response against *Lawsonia spp.*, and preferably against *L. intracellularis,* therein. As used herein, the phrase "induction of a protective immune response", and the like, refers to the ability of the administered polypeptide of the present invention to prevent or detectably slow the onset, development, or progression of symptoms associated with *Lawsonia* infection, and preferably, to prevent or detectably slow the onset, development, or progression of symptoms associated with PPE in pigs.

Preferably, the isolated or recombinant immunogenic polypeptide is selected from the group consisting of the following:

(i) a polypeptide which comprises an amino acid sequence which has at least about 60% sequence identity overall to an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, and 18;

(ii) a polypeptide which comprises an amino acid sequence which has at least about 60% sequence identity overall to an amino acid sequence encoded by *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM);

(iii) a polypeptide which comprises at least about 5 contiguous amino acids, preferably at least about 10 contiguous amino acids and more preferably at

least about 20 contiguous amino acids of an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, and 18;

(iv) a polypeptide which comprises at least about 5 contiguous amino acids of an amino acid sequence encoded by *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM); and

(v) a homologue, analogue or derivative of any one of (i) to (iv) which mimics a B-cell or T-cell epitope of *Lawsonia spp.*

In an alternative preferred embodiment, the isolated or recombinant immunogenic polypeptide is selected from the group consisting of the following:

(i) a polypeptide which comprises an amino acid sequence encoded by a nucleotide sequence having at least about 60% sequence identity overall to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17;

(ii) a polypeptide which comprises an amino acid sequence encoded by a nucleotide sequence having at least about 60% sequence identity overall to the nucleotide sequence of *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM);

(iii) a polypeptide encoded by at least about 15 contiguous nucleotides of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17;

(iv) a polypeptide encoded by at least about 15 contiguous nucleotides of a nucleotide sequence of *L. intracellularis* DNA contained within a plasmid

selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM); and

(v) a homologue, analogue or derivative of any one of (i) to (iv) which mimics a B-cell or T-cell epitope of *Lawsonia spp*.

Preferably, the immunogenic polypeptide encompassed by the present invention has at least about 70% identity, more preferably at least about 80% identity, even more preferably at least about 90% identity, and still even more preferably at least about 95% identity to the amino acid sequence of a polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides, as hereinbefore defined.

In determining whether or not two amino acid sequences fall within these percentage limits, those skilled in the art will be aware that it is necessary to conduct a side-by-side comparison or multiple alignment of sequences. In such comparisons or alignments, differences will arise in the positioning of non-identical residues, depending upon the algorithm used to perform the alignment. In the present context, reference to a percentage sequence identity or similarity between two or more amino acid sequences shall be taken to refer to the number of identical and similar residues respectively, between said sequences as determined using any standard algorithm known to those skilled in the art. For example, amino acid sequence identities or similarities may be calculated using the GAP programme of the Computer Genetics Group, Inc., University Research Park, Madison, Wisconsin, United States of America (Devereaux *et al*, 1984). The GAP programme utilizes the algorithm of Needleman and Wunsch (1970) to maximise the number of identical/similar residues and to minimise the number and/or length of sequence gaps in the alignment. Alternatively or in addition, where more than two amino acid sequences are being compared, the ClustalW programme of Thompson *et al* (1994) can be used.

Preferably, the isolated or recombinant immunogenic polypeptide of the invention comprises at least about 10 contiguous amino acids of a polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides, as hereinbefore defined. More preferably, the isolated or recombinant immunogenic polypeptide of the invention comprises at least about 20 contiguous amino acid residues of a polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides, as hereinbefore defined. Even more preferably, the isolated or recombinant immunogenic polypeptide of the invention comprises at least about 30 contiguous amino acid residues of a polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides, as hereinbefore defined, and still even more preferably, at least about 40 contiguous amino acid residues of said flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, or ytfN polypeptides.

The present invention further encompasses homologues, analogues and derivatives of a polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides, as hereinbefore defined.

"Homologues" of a polypeptide are those immunogenic polypeptides that are derived from a full-length *L. intracellularis* polypeptides described herein, or have sequence similarity to a full-length *L. intracellularis* polypeptide, notwithstanding one or more amino acid substitutions, deletions and/or additions relative to the full-length *L. intracellularis* polypeptide. A homologue may also retain the biological activity or catalytic activity of the full-length polypeptide. In such homologues, one or more amino acids can be replaced by other amino acids having similar properties such as, for example, hydrophobicity, hydrophilicity, hydrophobic moment, antigenicity, propensity to form or break α-helical structures of β-sheet structures, and so on.

Substitutional variants are those in which at least one residue in the sequence has been removed and a different residue inserted in its place. Amino acid substitutions

are typically of single residues, but may be clustered depending upon functional constraints placed upon the polypeptide; insertions will usually be of the order of about 1-10 amino acid residues. and deletions will range from about 1-20 residues. Preferably, amino acid substitutions will comprise conservative amino acid substitutions, such as those described *supra*.

Insertional amino acid sequence variants are those in which one or more amino acid residues are introduced into a predetermined site in the protein. Insertions can comprise amino-terminal and/or carboxyl terminal fusions as well as intra-sequence insertions of single or multiple amino acids. Generally, insertions within the amino acid sequence will be smaller than amino or carboxyl terminal fusions, of the order of about 1 to 4 residues.

Deletional variants are characterised by the removal of one or more amino acids from the sequence.

Amino acid variants of the polypeptide of the present invention may readily be made using polypeptide synthetic techniques well known in the art, such as solid phase synthesis and the like, or by recombinant DNA manipulations. The manipulation of DNA sequences to produce variant proteins which manifest as substitutional, insertional or deletional variants are well known in the art. For example, techniques for making substitution mutations at predetermined sites in DNA having known sequence are well known to those skilled in the art, such as by M13 mutagenesis or other site-directed mutagenesis protocol.

"Analogues" are defined as those immunogenic polypeptides that are derived from a full-length *L. intracellularis* polypeptides described herein, or have sequence similarity to a full-length *L. intracellularis* polypeptide; notwithstanding one or more non-naturally occurring or modified amino acid residues relative to the naturally-occurring full-length *L. intracellularis* polypeptide. The term "analogue" shall also be taken to include an amino acid sequence which is not similar to an amino acid sequence of a full-length

L. intracellularis polypeptide set forth herein, however mimics or cross-reacts with a B-cell or T-cell epitope of *Lawsonia spp.* and preferably, mimics or cross-reacts with a B-cell or T-cell epitope of *L. intracellularis,* such as, for example, a polypeptide which is derived from a computational prediction or empirical data revealing the secondary, tertiary or quaternary structure of the full-length polypeptide or an epitope thereof.

For example, mimotopes (polypeptide analogues that cross-react with a B-cell or T-cell epitope of the *Lawsonia* polypeptide of the invention but, however, comprise a different amino acid sequence to said epitope) may be identified by screening random amino acid sequences in polypeptide libraries with antibodies that bind to a desired T-cell or B-cell epitope. As with techniques for the identification of B-cell or T-cell epitopes as described *supra*, the antibodies used to identify such mimotopes may be polyclonal or monoclonal or recombinant antibodies, in crude or purified form. Mimotopes of a T-cell epitope may then be assayed further for their ability to stimulate T-cell cytotoxic or proliferative responses *in vitro.* Mimotopes are particularly useful as analogues of non-linear (i.e., conformational) epitopes of the polypeptide of the present invention, because conformational epitopes are generally formed from non-contiguous regions in a polypeptide, and the mimotopes provide immunogenic equivalents thereof in the form of a single polypeptide molecule.

Additionally, the use of polypeptide analogues can result in polypeptides with increased immunogenic and/or antigenic activity, that are less sensitive to enzymatic degradation, and which are more selective. A suitable proline analogue is 2-aminocyclopentane carboxylic acid (βAC^5c) which has been shown to increase the immunogenic activity of a native polypeptide more than 20 times (Mierke *et al*, 1990; Portoghese *et al*, 1990; Goodman *et al*, 1987).

"Derivatives" of a polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides, as hereinbefore defined, are those peptides or polypeptides which comprise at least about five contiguous amino acid residues of any one or more of said flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, or ytfN polypeptides.

A "derivative" may further comprise additional naturally-occurring, altered glycosylated, acylated or non-naturally occurring amino acid residues compared to the amino acid sequence of a flhB, or fliR, or ntrC, or glnH, or motA, or motB, or tlyC, or ytfM, or ytfN polypeptide, as hereinbefore defined. Alternatively or in addition, a derivative may comprise one or more non-amino acid substituents such as, for example, a reporter molecule or other ligand, covalently or non-covalently bound to the amino acid sequence of a flhB, or fliR, or ntrC, or glnH, or motA, or motB, or tlyC, or ytfM, or ytfN polypeptide, such as, for example, a reporter molecule which is bound thereto to facilitate its detection.

Other examples of recombinant or synthetic mutants and derivatives of a polypeptide immunogen of the present invention include those incorporating single or multiple substitutions in the amino acid sequence of a polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides. Recombinant or synthetic mutants and derivatives produced by making deletions from the amino acid sequence of a polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides, are also included within the scope of preferred derivatives. Additionally, recombinant or synthetic mutants and derivatives produced by making additions to a polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides, such as, for example, using carbohydrates, lipids and/or proteins or polypeptides, are also encompassed by the present invention.

Naturally-occurring or altered glycosylated or acylated forms of the flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, or ytfN polypeptides are particularly contemplated by the present invention.

Additionally, homopolymers or heteropolymers comprising one or more copies of the reference polypeptides, or one or more derivatives, homologues or analogues thereof, are clearly within the scope of the present invention.

Preferably, homologues, analogues and derivatives of the flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, or ytfN polypeptides of the invention are "immunogenic", defined hereinafter as the ability of said polypeptide, or a derivative, homologue or analogue thereof, to elicit B cell and/or T cell responses in the host, in response to immunization.

Preferred homologues, analogues and derivatives of the flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, or ytfN polypeptides of the invention include any amino acid variant that functions as B cell or T cell epitope of any one of said polypeptides, wherein said variant is capable of mediating an immune response, such as, for example, a mimotope of the immunogenic polypeptide which has been produced by synthetic means, such as by Fmoc chemistry. The only requirement of such variant molecules is that they cross-react immunologically with a polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN, as hereinbefore defined, or an epitope of said polypeptide.

As will be apparent to those skilled in the art, such homologues, analogues and derivatives of the polypeptides of the invention molecules will be useful to prepare antibodies that cross-react with antibodies against said polypeptide and/or to elicit a protective immune response of similar specificity to that elicited by said polypeptide. Such molecules will also be useful in diagnostic and other applications that are immunological in nature such as, for example, diagnostics which utilise one or more immunoassay formats (eg. ELISA, RIA and the like).

Accordingly, the immunogen of the present invention or a derivative, homologue or analogue thereof is useful in vaccine compositions that protect an individual against infection by *L. intracellularis* and/or as an antigen to elicit polyclonal or monoclonal antibody production and/or in the detection of antibodies against *L. intracellularis* in infected animals, particularly in porcine and avian animals.

The polypeptides of the present invention may comprise leader sequences to facilitate their secretion into the periplasmic space, either as part of the native protein, or alternatively, added by recombinant engineering means. Such may have improved

immunogenicity compared to non-secreted or non-secretable polypeptides of *L. intracellularis*, or non-secreted or non-secretable polypeptides of other causative agents of PPE. The particular advantages of such peptides will be immediately apparent to those skilled in the production of vaccine compositions, where the inherent immunogenicity of the immunogen is an important consideration for a protective immune response to be conferred.

Moreover, unique regions of the *L. intracellularis* polypeptides exemplified herein are promising antigenic peptides for the formulation of *Lawsonia*-specific vaccines and diagnostics for the specific detection of *Lawsonia spp.* in biological samples.

A second aspect of the present invention provides a vaccine composition for the prophylaxis or treatment of infection in a mammal or bird by *L. intracellularis* or similar or otherwise related microorganism, said vaccine composition comprising:

(i) an immunogenic component which comprises an isolated or recombinant polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides or an immunogenic homologue, analogue or derivative of any one of said polypeptides which is immunologically cross-reactive with *L. intracellularis;* and

(ii) one or more carriers, diluents and/or adjuvants suitable for veterinary or pharmaceutical use.

As used herein, the term "immunogenic component" refers to a polypeptide encoded by DNA from, or derived from, *L. intracellularis* or a related microorganism thereto which is capable of inducing a protective immune response in an animal, in particular a porcine or avian animal, whether or not said polypeptide is in an isolated or recombinant form. Accordingly, the vaccine composition clearly encompasses those vaccine compositions which comprise attenuated, killed or non-pathogenic isolates or forms of *L. intracellularis* or related microorganisms thereto which comprise or express said polypeptide.

By "protective immune response" is meant that the immunogenic component elicits an immune response in the animal to which the vaccine composition is administered at the humoral and/or cellular level which is sufficient to prevent infection by *L. intracellularis* or a related microorganism thereto and/or which is sufficient to detectably reduce one or more symptoms or conditions, or to detectably slow the onset of one or more symptoms or conditions, associated with infection by *L. intracellularis* or a related microorganism thereto in an animal host, as compared to a control infected animal. The term "effective amount" of an immunogenic component present in the vaccine composition refers to that amount of said immunogenic component that is capable of inducing a protective immune response after a single complete dose has been administered, or after several divided doses have been administered.

Preferably, the polypeptide component of the subject vaccine composition comprises an amino acid sequence which is both immunogenic and specific, by virtue of its immunological cross-reactivity with the causative agent of PPE, *L. intracellularis*. In this regard, it will be apparent from the preceding description that such polypeptide components may comprise the amino acid sequence of a polypeptide of *L. intracellularis* as exemplified herein, or alternatively, an immunologically cross-reactive homologue, analogue or derivative of said amino acid sequence, such as, for example, a mimotope of said sequence.

The immunogenic polypeptide or immunogenic homologue, analogue or derivative may be a naturally-occurring polypeptide in isolated or recombinant form according to any of the embodiments described *supra* or exemplified herein. Preferably, the immunogenic polypeptide or immunogenic homologue, analogue or derivative is derived from *Lawsonia spp.*, in particular *L. intracellularis* or a microorganism that is related thereto.

Preferably, the immunogenic component has undergone at least one purification step or at least partial concentration from a cell culture comprising *L. intracellularis* or a related microorganism thereto, or from a lysed preparation of *L. intracellularis* cells or

related microorganism, or from another culture in which the immunogenic component is recombinantly expressed. The purity of such a component which has the requisite immunogenic properties is preferably at least about 20% by weight of protein in a particular preparation, more preferably at least about 50%, even more preferably at least about 60%, still more preferably at least about 70% and even more preferably at least about 80% or greater.

The immunogenic component of the vaccine of the present invention can comprise a single polypeptide, or a range or combination of different polypeptides covering different or similar epitopes. In addition or, alternatively, a single polypeptide can be provided with multiple epitopes. The latter type of vaccine is referred to as a polyvalent vaccine. A multiple epitope includes two or more epitopes located within a polypeptide molecule.

The formulation of vaccines is generally known in the art and reference can conveniently be made to Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Co., Easton, Pennsylvania, USA.

A particularly useful form of the vaccine is a recombinant vaccine produced, for example, in a vaccine vector, such as but not limited to a mammalian cell transfected with a vaccinia virus vector, an insect cell transfected with a baculovirus vector, or a bacterial cell transfected with a plasmid or cosmid, the only requirement being that the vector expresses the immunogenic component.

The present invention clearly extends to recombinant vaccine compositions in which the immunogenic component at least is contained within killed vaccine vectors prepared, for example, by heat, formalin or other chemical treatment, electric shock or high or low pressure forces. According to this embodiment, the immunogenic component of the vaccine is generally synthesized in a live vaccine vector which is killed prior to administration to an animal.

Furthermore, the vaccine vector expressing the immunogenic component may be non-pathogenic or attenuated. Within the scope of this embodiment are cells that have been transfected with non-pathogenic or attenuated viruses encoding the immunogenic component of the vaccine and non-pathogenic or attenuated cells that directly express the immunogenic component.

Attenuated or non-pathogenic host cells include those cells which are not harmful to an animal to which the subject vaccine is administered. As will be known to those skilled in the art, "live vaccines" can comprise an attenuated virus vector encoding the immunogenic component or a host cell comprising same, which is capable of replicating in an animal to which it is administered, and using host cell machinery to express the immunogenic component albeit producing no adverse side-effects therein. Such vaccine vectors may colonise the gut or other organ of the vaccinated animal. Such live vaccine vectors are efficacious by virtue of their ability to continually express the immunogenic component in the host animal for a time and at a level sufficient to confer protective immunity against a pathogen which expresses an immunogenic equivalent of said immunogenic component. The present invention clearly encompasses the use of such attenuated or non-pathogenic vectors and live vaccine preparations.

The vaccine vector may be a virus, bacterial cell or a eukaryotic cell such as an insect, avian, porcine or other mammalian cell or a yeast cell or a cell line such as COS, VERO, HeLa, mouse C127, Chinese hamster ovary (CHO), WI-38, baby hamster kidney (BHK) or MDCK cell lines. Suitable prokaryotic cells include *Mycobacterium spp., Corynebacterium spp., Salmonella spp., Escherichia coli, Bacillus spp.* and *Pseudomonas spp*, amongst others. Bacterial strains which are suitable for the present purpose are well-known in the relevant art (Ausubel *et al*, 1987; Sambrook *et* al, 1989).

Such cells and cell lines are capable of expression of a genetic sequence encoding a polypeptide of the present invention from *L. intracellularis*, or a homologue, analogue

or derivative thereof, in a manner effective to induce a protective immune response in the animal. For example, a non-pathogenic bacterium can be prepared containing an expression vector which comprises a nucleotide sequence encoding a polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides, or a homologue, analogue, or derivative thereof, wherein said nucleotide sequence is placed operably under the control of a constitutive or inducible promoter sequence. The bacterium is then permitted to colonise suitable locations in a pig's gut, where it replicates and expresses the said polypeptide in amount sufficient to induce a protective immune response against *L. intracellularis*.

In a further alternative embodiment, the vaccine can be a DNA or RNA vaccine comprising a DNA or RNA molecule encoding a polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides or homologues, analogues or derivatives thereof, wherein said vaccine is injected into muscular tissue or other suitable tissue in a pig under conditions sufficient to permit transient expression of said DNA or RNA to produce an effective amount of said polypeptide to induce a protective immune response. In a preferred embodiment, the DNA vaccine is in the form of a plasmid, in which the DNA is operably connected with a promoter region capable of expressing the nucleotide sequence encoding the immunogen in cells of the immunized animal.

In the production of a recombinant vaccine, except for a DNA vaccine described herein, it is therefore necessary to express the immunogenic component in a suitable vector system. For the present purpose, the immunogenic component can be expressed by:

(i) placing an isolated nucleic acid molecule in an expressible format, said nucleic acid molecule comprising the coding region of a gene selected from the group consisting of *flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM*, and *ytfN* genes, or a protein-encoding homologue, analogue or derivative thereof;

(ii) introducing the isolated nucleic acid molecule of (i) in an expressible format into a suitable vaccine vector; and

(iii) incubating or growing the vaccine vector for a time and under conditions sufficient for expression of the immunogenic component encoded by said nucleic acid molecule to occur.

It will be apparent from the preceding discussion that the protein-encoding region of a *flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM*, or *ytfN* gene comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, and 17, or alternatively or in addition, a protein-encoding nucleotide sequence of *L. intracellularis* DNA contained within a deposited plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM).

Preferred homologues of the protein-encoding region of a *flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM*, or *ytfN* gene include those nucleotide sequences selected from the group consisting of:

(i) a protein-encoding nucleotide sequence having at least about 60% sequence identity overall to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17 or a degenerate variant thereof;

(ii) a protein-encoding nucleotide sequence having at least about 60% sequence identity overall to the protein-encoding sequence of *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM);

(iii) a protein-encoding nucleotide sequence which comprises at least about 15 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17;

(iv) a protein-encoding nucleotide sequence which comprises at least about 15 contiguous nucleotides of the protein-encoding sequence of *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM);

(v) a protein-encoding nucleotide sequence which hybridizes under at least low stringency conditions to the complement of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17; and

(vi) a protein-encoding nucleotide sequence which hybridizes under at least low stringency conditions to the non-coding strand of *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM).

The present invention clearly extends to analogues or derivatives of any one of (i) to (vi) which encode a polypeptide which mimics a B-cell or T-cell epitope of *Lawsonia spp*.

For the present purpose, a preferred homologue of the protein-encoding region of a *flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM*, or *ytfN* gene will have at least about 80% nucleotide sequence identity to the coding region of said gene, still more preferably at least about 90% identity, and yet still more preferably at least about 95% identity.

In determining whether or not two nucleotide sequences fall within these percentage limits, those skilled in the art will be aware that it is necessary to conduct a side-by-side comparison or multiple alignment of sequences. In such comparisons or alignments, differences may arise in the positioning of non-identical residues, depending upon the

algorithm used to perform the alignment. In the present context, reference to a percentage identity between two or more nucleotide sequences shall be taken to refer to the number of identical residues between said sequences as determined using any standard algorithm known to those skilled in the art. For example, nucleotide sequences may be aligned and their identity calculated using the BESTFIT programme or other appropriate programme of the Computer Genetics Group, Inc., University Research Park, Madison, Wisconsin, United States of America (Devereaux *et al*, 1984).

Preferably, a homologue of the protein-encoding region of a *flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM*, or *ytfN* gene hybridizes under at least medium stringency conditions to the non-coding strand of said gene, even more preferably under high stringency conditions to the non-coding strand of said gene.

For the purposes of defining the level of stringency, a low stringency is defined herein as being a hybridisation and/or a wash carried out in 6xSSC buffer, 0.1% (w/v) SDS at 28°C. A moderate stringency is defined herein as being a hybridisation and/or washing carried out in 2xSSC buffer, 0.1% (w/v) SDS at a temperature in the range 45°C to 65°C. A high stringency is defined herein as being a hybridisation and/or wash carried out in 0.1xSSC buffer, 0.1% (w/v) SDS, or lower salt concentration, and at a temperature of at least 65°C. Reference herein to a particular level of stringency encompasses equivalent conditions using wash/hybridization solutions other than SSC known to those skilled in the art.

Generally, the stringency is increased by reducing the concentration of SSC buffer, and/or increasing the concentration of SDS and/or increasing the temperature of the hybridisation and/or wash. Those skilled in the art will be aware that the conditions for hybridisation and/or wash may vary depending upon the nature of the hybridisation membrane or the type of hybridisation probe used. Conditions for hybridisations and washes are well understood by one normally skilled in the art. For the purposes of clarification of the parameters affecting hybridisation between nucleic acid molecules,

reference is found in pages 2.10.8 to 2.10.16. of Ausubel *et al.* (1987), which is herein incorporated by reference.

As used herein, a "nucleic acid molecule in an expressible format" is a protein-encoding region of a nucleic acid molecule placed in operable connection with a promoter or other regulatory sequence capable of regulating expression in the vaccine vector system.

Reference herein to a "promoter" is to be taken in its broadest context and includes the transcriptional regulatory sequences of a classical genomic gene, including the TATA box which is required for accurate transcription initiation, with or without a CCAAT box sequence and additional regulatory elements (i.e., upstream activating sequences, enhancers and silencers) which alter gene expression in response to developmental and/or external stimuli, or in a tissue-specific manner. In the present context, the term "promoter" is also used to describe a recombinant, synthetic or fusion molecule, or derivative which confers, activates or enhances the expression of a nucleic acid molecule to which it is operably connected, and which encodes the immunogenic polypeptide. Preferred promoters can contain additional copies of one or more specific regulatory elements to further enhance expression and/or to alter the spatial expression and/or temporal expression of the said nucleic acid molecule.

Placing a nucleic acid molecule under the regulatory control of, i.e., "in operable connection with", a promoter sequence means positioning said molecule such that expression is controlled by the promoter sequence. Promoters are generally, but not necessarily, positioned 5′ (upstream) to the genes that they control. In the construction of heterologous promoter/structural gene combinations it is generally preferred to position the promoter at a distance from the gene transcription start site that is approximately the same as the distance between that promoter and the gene it controls in its natural setting, i.e., the gene from which the promoter is derived. Furthermore, the regulatory elements comprising a promoter are usually positioned within 2 kb of the start site of transcription of the gene. As is known in the art, some variation in this distance can be accommodated without loss of promoter function. Similarly, the preferred positioning of a regulatory sequence element with respect to a heterologous gene to be placed under its control is defined by the positioning of the element in its natural setting, i.e., the genes from which it is derived. Again, as is known in the art, some variation in this distance can also occur.

The prerequisite for producing intact polypeptides in bacteria such as *E. coli* is the use of a strong promoter with an effective ribosome binding site. Typical promoters suitable for expression in bacterial cells such as *E. coli* include, but are not limited to, the *lacz* promoter, temperature-sensitive λ_L or λ_R promoters, T7 promoter or the IPTG-inducible *tac* promoter. A number of other vector systems for expressing the nucleic acid molecule of the invention in *E. coli* are well-known in the art and are described, for example, in Ausubel *et al* (1987) or Sambrook *et al* (1989). Numerous plasmids with suitable promoter sequences for expression in bacteria and efficient ribosome binding sites have been described, such as for example, pKC30 (λ_L: Shimatake and Rosenberg, 1981); pKK173-3 (*tac*: Amann and Brosius, 1985), pET-3 (T7: Studier and Moffat, 1986); the pBAD/TOPO or pBAD/Thio-TOPO series of vectors containing an arabinose-inducible promoter (Invitrogen, Carlsbad, CA), the latter of which is designed to also produce fusion proteins with thioredoxin to enhance solubility of the expressed protein; the pFLEX series of expression vectors (Pfizer Inc., CT, USA); or the pQE series of expression vectors (Qiagen, CA), amongst others. Typical promoters suitable

for expression in viruses of eukaryotic cells and eukaryotic cells include the SV40 late promoter, SV40 early promoter and cytomegalovirus (CMV) promoter, CMV IE (cytomegalovirus immediate early) promoter amongst others.

Means for introducing the isolated nucleic acid molecule or a genetic construct comprising same into a cell for expression of the immunogenic component of the vaccine composition are well-known to those skilled in the art. The technique used for a given organism depends on the known successful techniques. Means for introducing recombinant DNA into animal cells include microinjection, transfection mediated by DEAE-dextran, transfection mediated by liposomes such as by using lipofectamine (Gibco, MD, USA) and/or cellfectin (Gibco, MD, USA), PEG-mediated DNA uptake, electroporation and microparticle bombardment such as by using DNA-coated tungsten or gold particles (Agracetus Inc., WI, USA) amongst others.

The immunogenic component of a vaccine composition as contemplated herein exhibits excellent therapeutic activity, for example, in the treatment and/or prophylaxis of PPE when administered in an amount which depends on the particular case. For example, for recombinant polypeptide molecules, from about 0.5 μg to about 20 mg may be administered, preferably from about 1 μg to about 10 mg, more preferably from about 10 μg to about 5 mg, and most preferably from about 50 μg to about 1 mg equivalent of the immunogenic component in a volume of about 1ml to about 5ml. For DNA vaccines, a preferred amount is from about 0.1 μg/ml to about 5 mg/ml in a volume of about 1 to about 5 ml. The DNA can be present in "naked" form or it can be administered together with an agent facilitating cellular uptake (e.g., in liposomes or cationic lipids). The important feature is to administer sufficient immunogen to induce a protective immune response. The above amounts can be administered as stated or calculated per kilogram of body weight. Dosage regime can be adjusted to provide the optimum therapeutic response. For example, several divided doses can be administered or the dose can be proportionally reduced as indicated by the exigencies of the therapeutic situation. Booster administration may also be required. The vaccine of the present invention can further comprise one or more additional

immunomodulatory components such as, for example, an adjuvant or cytokine molecule, amongst others, that is capable of increasing the immune response against the immunogenic component. Non-limiting examples of adjuvants that can be used in the vaccine of the present invention include the RIBI adjuvant system (Ribi Inc., Hamilton, MT, USA), alum, mineral gels such as aluminium hydroxide gel, oil-in-water emulsions, water-in-oil emulsions such as, for example, Block co-polymer (CytRx, Atlanta GA, USA),QS-21 (Cambridge Biotech Inc., Cambridge MA, USA), SAF-M (Chiron, Emeryville CA, USA), AMPHIGEN® adjuvant, Freund's complete adjuvant; Freund's incomplete adjuvant; and Saponin, QuilA or other saponin fraction, monophosphoryl lipid A, and Avridine lipid-amine adjuvant. Other immunomodulatory agents that can be included in the vaccine include, for example, one or more cytokines, such as interferon and/or interleukin, or other known cytokines. Non-ionic surfactants such as, for example, polyoxyethylene oleyl ether and n-hexadecyl polyethylene ether may also be included in the vaccines of the present invention.

The vaccine composition can be administered in a convenient manner such as by oral, intravenous (where water soluble), intramuscular, subcutaneous, intranasal, intradermal or suppository routes or by implantation (eg., using slow release technology), provided that a sufficient degree of the immunogenicity of the immunizing antigen is retained for the purposes of eliciting an immune response in the animal being treated. Depending on the route of administration, the immunogenic component may be required to be coated in a material to protect it from the action of enzymes, acids and other natural conditions which may inactivate it, such as those in the digestive tract.

The vaccine composition may also be administered parenterally or intraperitoneally. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof, or in oils. Under ordinary conditions of storage and use, these preparations can contain a preservative to prevent the growth of microorganisms. Alternatively, the vaccine composition can be stored in lyophilised form to be rehydrated with an appropriate vehicle or carrier prior to use.

Pharmaceutical forms suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. In all cases the form must be fluid to the extent that easy syringability exists, unless the pharmaceutical form is a solid or semi-solid such as when slow release technology is employed. In any event, it must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms.

The carrier may be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol and liquid polyethylene glycol, and the like), suitable mixtures thereof and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents such as, for example,, parabens, chlorobutanol, phenol, sorbic acid, thimerosal and the like. In many cases, it will be preferable to include isotonic agents such as, for example,, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption such as, for example,, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compound in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filter-sterilization. Generally, dispersions are prepared by incorporating the sterilized active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients selected from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solution thereof.

The present invention extends to vaccine compositions which confer protection against infection by one or more isolates or sub-types of *L. intracellularis* including those that belong to the same serovar or serogroup as *L. intracellularis*. The vaccine composition preferably also confers protection against infection by other species of the genus *Lawsonia* or other microorganisms related thereto, as determined at the nucleotide, biochemical, structural, physiological and/or immunointeractive level; the only requirement being that said other species or other microorganism expresses a polypeptide which is immunologically cross-reactive to a polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides, or a homologue, analogue or derivative of any one or more of said polypeptides as described herein. For example, such related microorganisms may comprise genomic DNA which is at least about 70% identical overall to the genomic DNA of *L. intracellularis* as determined using standard genomic DNA hybridisation and analysis techniques.

The terms "serogroup" and "serovar" relate to a classification of microorganisms which is based upon serological typing data, in particular data obtained using agglutination assays such as the microscopic agglutination test (MAT). Those skilled in the art will be aware that serovar and serogroup antigens are a mosaic on the cell surface and, as a consequence there will be no strict delineation between bacteria belonging to a serovar and/or serogroup. Moreover, organisms which belong to different species may be classified into the same serovar or serogroup because they are indistinguishable by antigenic determination. As used herein, the term "serovar" means one or more *Lawsonia* strains which are antigenically-identical with respect to antigenic determinants produced by one or more loci. Quantitatively, serovars may be differentiated from one another by cross-agglutination absorption techniques. As used herein, the term "serogroup" refers to a group of *Lawsonia spp.* whose members cross-agglutinate with shared group antigens and do not cross-agglutinate with the members of other groups and, as a consequence, the members of a serogroup have more or less close antigenic relations with one another by simple cross-agglutination.

The present invention thus clearly extends to vaccine compositions for the treatment and/or prophylaxis of animals, in particular, vaccine compositions for the treatment and/or prophylaxis of porcine and/or avian species, against any bacterium belonging to the same serovar or serogroup as *L. intracellularis.* Preferably, such organisms will express a polypeptide homologue, analogue or derivative of a polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides.

The present invention extends further to vaccine compositions capable of conferring protection against a "genetic variant" of *L. intracellularis*, the only requirement being that said variant expresses a polypeptide which is immunologically cross-reactive to a polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides. Genetic variants of *L. intracellularis* can be developed by mutation, recombination, conjugation or transformation of *L. intracellularis* or may occur naturally. It will be known to a person skilled in the art how to produce such derivatives.

In a particularly preferred embodiment, the vaccine composition of the invention is intended for or suitable for the prophylaxis and/or treatment of infection in a porcine or avian animal and more preferably, for prophylaxis and/or treatment of a porcine animal for infection by *L. intracellularis.*

Accordingly, the present invention clearly extends to the use of the immunogenic polypeptide of the invention or a DNA or RNA molecule encoding the same, according to any one of the preceding embodiments or as exemplified herein in the preparation of a medicament for the treatment and/or prophylaxis of PPE in animals, particularly porcine or avian animals.

The invention further extends to a method of treatment and/or prophylaxis of PPE in an animal such as an avian or porcine animal, said method comprising administering

the vaccine composition or the immunogenic polypeptide of the invention or a DNA or RNA molecule encoding the same, as described or exemplified herein to said animal for a time and under conditions sufficient for an immune response to occur thereto. Preferably, in the case of administration of a vaccine composition, the immune response to the immunogen is a protective immune response.

Those skilled in the art will recognise the general applicability of the invention in vaccinating animals other than porcine and avian animals against *L. intracellularis* and/or related microorganisms. In the general application of the vaccine of the present invention, the only prerequisite is that the animal on which protection is conferred is capable of being infected with *L. intracellularis* and/or a related microorganism thereto and that, in the case of a related microorganism to *L. intracellularis,* said related microorganism expresses a B-cell or T-cell epitope which mimics or cross-reacts with the polypeptide component of the vaccine composition described herein. Animals which may be protected by the vaccine of the present invention include, but are not limited to, humans, primates, companion animals (e.g., cats, dogs), livestock animals (e.g., pigs, sheep, cattle, horses, donkeys, goats), laboratory test animals (e.g., mice, rats, guinea pigs, rabbits) and captive wild animals (e.g., kangaroos, foxes, deer). The present invention also extends to the vaccination of birds such as poultry birds, game birds and caged birds.

The present invention further extends to combination vaccines comprising an effective amount of a first immunogenic component comprising a polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides, or a homologue, analogue or derivative thereof as described herein, or a DNA or RNA molecule encoding the same, combined with an effective amount of a second immunogenic component comprising one or more other antigens capable of protecting a porcine animal, or bird, against either *Lawsonia spp.* or another pathogen that infects and causes disease in said animal. The second immunogenic component is different from the first immunogenic component and is preferably selected from the group consisting of the *L. intracellularis* FlgE, hemolysin, OmpH, SodC, flhB, fliR, ntrC, glnH,

motA, motB, tlyC, ytfM, and ytfN polypeptides and homologues, analogues or derivatives thereof. The present invention clearly extends to DNA vaccines and vaccine vectors which express said first immunogenic component and said second immunogenic component.

It is within the scope of the invention to encompass vaccine compositions comprising multimeric and polymeric forms of any one or more of the immunogenic polypeptides described herein, such as tandem arrays of homologous amino acid sequences, or, alternatively, tandem arrays of heterologous immunogenic repeats of amino acid sequences. The present invention extends further to nucleic acid molecules encoding such polymeric forms.

The isolated or recombinant polypeptide of the invention, or an immunologically-equivalent homologue, analogue or derivative thereof is also useful for the preparation of immunologically interactive molecules which are useful in the diagnosis of infection of an animal by *Lawsonia spp.*, in particular by *L. intracellularis* or a related organism thereto.

As used herein, the term "immunologically interactive molecule" includes antibodies and antibody derivatives and functional equivalents, such as a Fab, or a SCAB (single-chain antibody), any of which optionally can be conjugated to an enzyme, radioactive or fluorescent tag, amongst others. The only requirement of such immunologically interactive molecules is that they are capable of binding specifically to the immunogenic polypeptide of the present invention as hereinbefore described.

Accordingly, a further aspect of the invention extends to an immunologically interactive molecule which is capable of binding to a polypeptide selected from the group consisting of:

(i) a polypeptide which comprises an amino acid sequence which has at least about 60% sequence identity overall to an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, and 18;

(ii) a polypeptide which comprises an amino acid sequence which has at least about 60% sequence identity overall to an amino acid sequence encoded by *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM);

(iii) a polypeptide which comprises at least about 5 contiguous amino acids of an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, and 18;

(iv) a polypeptide which comprises at least about 5 contiguous amino acids of an amino acid sequence encoded by *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM);

(v) a polypeptide which comprises an amino acid sequence encoded by a nucleotide sequence having at least about 60% sequence identity overall to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17;

(vi) a polypeptide which comprises an amino acid sequence encoded by a nucleotide sequence having at least about 60% sequence identity overall to the nucleotide sequence of *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM);

(vii) a polypeptide encoded by at least about 15 contiguous nucleotides of a

nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17;

(viii) a polypeptide encoded by at least about 15 contiguous nucleotides of a nucleotide sequence of *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM); and

(ix) a homologue, analogue or derivative of any one of (i) to (viii) which mimics a B-cell or T-cell epitope of *Lawsonia spp.*

In a preferred embodiment, the immunologically interactive molecule is an antibody that binds specifically to one or more epitopes of a polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides. More preferably, the immunologically interactive molecule binds specifically to one or more epitopes of a polypeptide from a causative agent of PPE, such as, for example, *L. intracellularis*.

Conventional methods can be used to prepare the immunologically interactive molecules. For example, by using a polypeptide immunogen of the present invention, polyclonal antisera or monoclonal antibodies can be made using standard methods. For example, a mammal, (e.g.,.a mouse, hamster, or rabbit) can be immunized with an immunogenic form of the polypeptide of the present invention which elicits an antibody response in the mammal. Techniques for conferring immunogenicity on a polypeptide include conjugation to carriers, or other techniques well known in the art. For example, the polypeptide can be administered in the presence of adjuvant or can be coupled to a carrier molecule, as known in the art, that enhances the immunogenicity of the polypeptide. The progress of immunization can be monitored by detection of antibody titres in plasma or serum. Standard ELISA or other immunoassay can be used with the immunogen as antigen to assess the levels of

antibodies. Following immunization, antisera can be obtained and, for example, IgG molecules corresponding to the polyclonal antibodies can be isolated from the antisera.

To produce monoclonal antibodies, antibody producing cells (lymphocytes) can be harvested from an animal immunised with a polypeptide of the present invention and fused with myeloma cells by standard somatic cell fusion procedures, thus immortalizing these cells and yielding hybridoma cells. Such techniques are well known in the art, for example, the hybridoma technique originally developed by Kohler and Milstein (1975), as well as other techniques such as the human B-cell hybridoma technique (Kozbor *et al.*, 1983), the EBV-hybridoma technique to produce human monoclonal antibodies (Cole *et al.*, 1985), and screening of combinatorial antibody libraries (Huse *et al.*, 1989). Hybridoma cells can be isolated and screened immunochemically for production of antibodies that are specifically reactive with the polypeptide and monoclonal antibodies isolated therefrom.

As with all immunogenic compositions for eliciting antibodies, the immunogenically effective amounts of the peptides of the invention must be determined empirically. Factors to be considered include the immunogenicity of the native polypeptide, whether or not the polypeptide will be complexed with or covalently attached to an adjuvant or carrier protein or other carrier, the route of administration for the composition, i.e., intravenous, intramuscular, subcutaneous, *etc.*, and the number of immunizing doses to be administered. Such factors are known in the vaccine art and it is well within the skill of immunologists to make such determinations without undue experimentation.

The term "antibody" as used herein, is intended to include fragments thereof which are also specifically reactive with a polypeptide that mimics or cross-reacts with a B-cell or T-cell epitope of the *L. intracellularis* polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides. Antibodies can be fragmented using conventional techniques and the fragments screened for utility

in the same manner as described above for whole antibodies. For example, F(ab')2 fragments can be generated by treating antibody with pepsin. The resulting F(ab')2 fragment can be treated to reduce disulfide bridges to produce Fab' fragments.

It is within the scope of this invention to include any secondary antibodies (monoclonal, polyclonal or fragments of antibodies), including anti-idiotypic antibodies, directed to the first mentioned antibodies discussed above. Both the first and second antibodies can be used in detection assays or a first antibody can be used with a commercially available anti-immunoglobulin antibody. An antibody as contemplated herein includes any antibody specific to any region of a polypeptide which mimics, or cross-reacts with a B-cell or T-cell epitope of a *L. intracellularis* polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides.

The antibodies described herein are useful for determining B-cell or T-cell epitopes of a polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides, such as, for example, by testing the ability of synthetic peptides to cross-react immunologically with said polypeptide or to elicit the production of antibodies which cross-react with said polypeptide. Using methods described herein, polyclonal antibodies, monoclonal antibodies or chimeric monoclonal antibodies can also be raised to peptides which mimic or cross-react with a B-cell or T-cell epitope of a *L. intracellularis* polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides.

More particularly, the polyclonal, monoclonal or chimeric monoclonal antibodies can be used to detect the polypeptide of the invention and/or any homologues, analogues or derivatives thereof, in various biological materials. For example, they can be used in an ELISA, radioimmunoassay, or histochemical test. In other words, the antibodies can be used to test for binding to a polypeptide of the invention or to a homologue, analogue or derivative thereof, in a biological sample to diagnose the presence of *L. intracellularis* therein.

Accordingly, a further aspect of the invention provides a method of diagnosing infection of an animal by *L. intracellularis* or a related microorganism thereto, said method comprising the steps of contacting a biological sample derived from said animal with an immunologically interactive molecule which is capable of binding to a polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides, or a homologue, analogue or derivative thereof, for a time and under conditions sufficient for an antigen:antibody complex to form, and detecting said complex formation.

According to this embodiment of the present invention, the immunologically interactive molecule is preferably an antibody molecule prepared against a *L. intracellularis* polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides, or an analogue or derivative thereof.

If the biological sample being tested contains one or more epitopes of a polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides, or an immunologically cross-reactive homologue, analogue or derivative thereof, it will give a positive binding result to the immunologically interactive molecule.

Preferably, the biological sample is derived from a porcine or avian host of the pathogen *L. intracellularis* or a related microorganism thereto, and includes an appropriate tissue or fluid sample from the animal.

Preferred biological samples are derived from the ileum, caecum, small intestine, large intestine, whole serum or lymph nodes of the porcine or avian host animal being tested. Alternatively or in addition the biological test sample may comprise faeces or a rectal swab derived from the animal.

To distinguish *L. intracellularis* from other microorganisms resident in the gut or other organ of an animal, the antibodies should not be prepared against highly-conserved

epitopes of the *L. intracellularis* polypeptide, such as, for example, those amino acid sequences of at least 5 amino acids in length which are conserved between *L. intracellularis* and a microorganism which is present in the gut or other organ of an animal in respect of which diagnosis is sought such as, for example, *E.coli.*

Conventional immunoassays can be used to perform this embodiment of the invention. A wide range of immunoassay techniques are available as can be seen by reference to US Patent Nos. 4,016,043, 4,424,279 and 4,018,653. These, of course, include both single-site and two-site or "sandwich" assays of the non-competitive types, as well as the traditional competitive binding assays. These assays also include direct binding of a labelled antibody to a target. It will be readily apparent to the skilled technician how to modify or optimise such assays to perform this embodiment of the present invention, and all such modifications and optimisations are encompassed by the present invention.

In one alternative embodiment, the present invention contemplates a method of identifying whether or not an animal has suffered from a past infection, or is currently infected with *L. intracellularis* or a related microorganism thereto, said method comprising contacting blood or serum derived from said animal with the immunogenic polypeptide of the invention for a time and under conditions sufficient for an antigen:antibody complex to form, and detecting said complex formation. This embodiment differs from the embodiment described *supra* in that it relies upon the detection of circulating antibodies against *L. intracellularis* or related organism in the animals blood or serum which are present as a consequence of a past or present infection by this pathogen. However, it will be apparent to those skilled in the art that the principle of the assay format is the same. As with other embodiments of the invention referred to *supra*, conventional immunoassays can be used. Persons skilled in the art will readily be capable of varying known immunoassay formats to perform the present embodiment. This embodiment of the invention can also utilise derivatives of blood and serum which comprise immunologically interactive molecules such as, for example, partially-purified IgG or IgM fractions and buffy coat samples, amongst

others. The preparation of such fractions will also be known to those skilled in the art.

A further aspect of the present invention provides an isolated nucleic acid molecule which comprises a sequence of nucleotides that encodes, or is complementary to a nucleic acid molecule that encodes a polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides, including any and all genes selected from the group consisting of *flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM*, and *ytfN* genes as defined hereinabove.

In a preferred embodiment, the isolated nucleic acid molecule comprises a nucleotide sequence encoding a polypeptide that is immunologically cross-reactive with *L. intracellularis* or other causative agent of PPE, wherein said nucleotide sequence is selected from the group consisting of:

(i) a nucleotide sequence having at least about 60% sequence identity overall to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17 or a degenerate variant thereof;

(ii) a nucleotide sequence having at least about 60% sequence identity overall to *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM); or a degenerate variant thereof;

(iii) a nucleotide sequence which comprises at least about 15 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17;

(iv) a nucleotide sequence which comprises at least about 15 contiguous nucleotides of *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5

tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM);

(v) a nucleotide sequence which hybridizes under at least low stringency conditions to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17 or a complementary nucleotide sequence thereto;

(vi) a nucleotide sequence which hybridizes under at least low stringency conditions to *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM); and

(vii) a homologue, analogue or derivative of any one of (i) to (vi) which encodes a polypeptide which mimics a B-cell or T-cell epitope of *Lawsonia spp.*

For the present purpose, a "homologue" of a nucleotide sequence shall be taken to refer to an isolated nucleic acid molecule which encodes a polypeptide that is immunologically cross-reactive to a polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides, but which includes one or more nucleotide substitutions, insertions, deletions, or rearrangements.

An "analogue" of a nucleotide sequence set forth herein shall be taken to refer to an isolated nucleic acid molecule which encodes a polypeptide which is immunologically cross-reactive to a polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides, but which includes one or more non-nucleotide constituents not normally present in said isolated nucleic acid molecule, such as, for example, carbohydrates, radiochemicals including radio nucleotides, reporter molecules such as, but not limited to biotin, DIG, alkaline phosphatase or horseradish peroxidase, amongst others.

A "derivative" of a nucleotide sequence set forth herein shall be taken to refer to any isolated nucleic acid molecule which contains at least about 60% nucleotide sequence identity to 15 or more contiguous nucleotides present in the nucleotide sequence of a gene selected from the group consisting of *flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM,* and *ytfN* genes.

Generally, a *flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM,* or *ytfN* gene may be subjected to mutagenesis to produce single or multiple nucleotide substitutions, deletions and/or insertions. Nucleotide insertional derivatives include 5′ and 3′ terminal fusions as well as intra-sequence insertions of single or multiple nucleotides or nucleotide analogues. Insertional nucleotide sequence variants are those in which one or more nucleotides or nucleotide analogues are introduced into a predetermined site in the nucleotide sequence of the gene, although random insertion is also possible with suitable screening of the resulting product being performed. Deletional nucleotide sequence variants are characterised by the removal of one or more nucleotides from the gene. Substitutional nucleotide sequence variants are those in which at least one nucleotide in the gene sequence has been removed and a different nucleotide or nucleotide analogue inserted in its place. In a preferred embodiment, such substitutions are selected based on the degeneracy of the genetic code, as known in the art, with the resulting substitutional variant encoding the amino acid sequence of a flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, or ytfN polypeptide.

Preferred homologues, analogues and derivatives of a *flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM,* or *ytfN* gene comprise a sequence of nucleotides which has at least about 80%identity, even more preferably at least about 90% identity, and yet still more preferably at least about 95% identity to said gene.

In determining whether or not two nucleotide sequences fall within these percentage limits, reference is made to the description *supra* of methods for conducting a side-by-side comparison or multiple alignment of nucleotide sequences.

Alternatively or in addition, preferred homologues, analogues and derivatives of a *flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM*, or *ytfN* gene comprise a sequence of nucleotides which hybridizes under at least moderate stringency conditions and to the nucleotide sequence of said gene, or to a nucleic acid fragment comprising at least about 20 contiguous nucleotides in length derived therefrom, and even more preferably, under high stringency conditions to said gene, or to said nucleic acid fragment. For the purposes of defining the level of stringency, reference is made to the description hereinabove of hybridization stringencies.

In a more preferred embodiment, such a nucleotide sequence encodes a polypeptide that is immunologically cross-reactive with *L. intracellularis* or other causative agent of PPE.

In a particularly preferred embodiment, the isolated nucleic acid molecule of the present invention comprises or consists of a nucleotide sequence selected from the group consisting of:

(i) a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1,3, 5, 7, 9, 11, 13, 15, and 17;

(ii) a nucleotide sequence of the *L. intracellularis* DNA contained within a deposited plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM); and

(iii) a nucleotide sequence that encodes the same polypeptide as (i) or (ii), wherein said polypeptide is selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides; and

(iv) a nucleotide sequence that is complementary to (i) or (ii) or (iii).

The present invention clearly encompasses genetic constructs comprising the subject nucleic acid molecule in an expressible format suitable for the preparation of a

recombinant immunogenic polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides, such as for use in recombinant univalent or polyvalent recombinant vaccines.

In such cases, the nucleic acid molecule will be operably connected to a promoter sequence which can thereby regulate expression of said nucleic acid molecule in a prokaryotic or eukaryotic cell as described *supra*.

The genetic construct optionally further comprises a terminator sequence. The term "terminator" refers to a DNA sequence at the end of a transcriptional unit which signals termination of transcription. A "terminator" is a nucleotide sequence, generally located within the 3′-non-translated region of a gene or mRNA, comprising a polyadenylation signal to facilitate the post-transcriptional addition of a polyadenylate sequence to the 3′-end of a primary mRNA transcript. Terminator sequences may be isolated from the genetic sequences of bacteria, fungi, viruses, animals and/or plants. Terminators active in animal cells are known and described in the literature.

In a preferred embodiment, the genetic construct can be a cloning or expression vector, as known in the art, such as a plasmid, cosmid, or phage, comprising a nucleic acid molecule of the present invention, and host cells transformed or transfected therewith. In a non-limiting embodiment, the vector is a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM).

The genetic constructs of the present invention are particularly useful for producing the immunogenic component of the vaccine composition described herein or for use in a DNA vaccine.

A range of genetic diagnostic assays to detect infection of an animal by *L.*

intracellularis or a related microorganism can be employed using the nucleic acid molecule described herein such as, for example, assays based upon the polymerase chain reaction (PCR) and nucleic acid hybridisation. All such assays are contemplated in the present invention.

Accordingly, a still further aspect of the invention provides a diagnostic method of detecting *L. intracellularis* or related microorganism in a biological sample derived from an animal subject, said method comprising the steps of hybridising one or more probes or primers derived from a nucleotide sequence of a *flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM*, or *ytfN* gene as defined hereinabove, or a homologue, analogue or derivative thereof, to a DNA or RNA molecule present in said sample and then detecting said hybridisation using a detection means.

As used herein, the term "probe" refers to a nucleic acid molecule which is capable of being used in the detection of a gene selected from the group consisting of *flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM*, and *ytfN* genes. Probes may comprise DNA (single-stranded or double-stranded) or RNA (i.e., riboprobes) or analogues thereof.

The term "primer" refers to a probe as hereinbefore defined which is further capable of being used to amplify a nucleotide sequence from *L. intracellularis* or a related microorganism thereto in a PCR.

Preferred probes and primers include fragments of a gene selected from the group consisting of *flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM*, and *ytfN* genes, including synthetic single-stranded DNA or RNA molecules of at least about 15 nucleotides in length.

Preferably, probes and primers according to this embodiment will comprise at least about 20 contiguous nucleotides in length from a gene selected from the group consisting of *flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM*, and *ytfN* genes, even more preferably at least about 25 contiguous nucleotides, still even more preferably at least

about 50 contiguous nucleotides, and even more preferably at least about 100 nucleotides to about 500 nucleotides in length from said gene. Probes and primers comprising the full-length gene or a complementary nucleotide sequence thereto are also encompassed by the present invention.

Probes or primers can comprise inosine, adenine, guanine, thymidine, cytidine or uracil residues or functional analogues or derivatives thereof that are capable of being incorporated into a polynucleotide molecule, provided that the resulting probe or primer is capable of hybridising under at least low stringency conditions to a gene selected from the group consisting of *flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM*, and *ytfN* genes, or is at least about 60% identical to one strand of said gene.

The biological sample according to this aspect of the invention includes any organ, tissue, cell or exudate which contains or is likely to contain *L. intracellularis* or a nucleic acid derived therefrom. A biological sample can be prepared in a suitable solution such as, for example, an extraction buffer or suspension buffer. The present invention extends to the testing of biological solutions thus prepared, the only requirement being that said solution at least comprises a biological sample as described herein.

The diagnostic assay of the present invention is useful for the detection of *L. intracellularis* or a microorganism which is related thereto which expresses a polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides.

The present invention clearly contemplates diagnostic assays which are capable of both genus-specific and species-specific detection. Accordingly, in one embodiment, the probe or primer, or a homologue, analogue or derivative thereof, comprises DNA capable of being used to detect multiple *Lawsonia spp.* In an alternative embodiment, the probe or primer or a homologue, analogue or derivative thereof comprises DNA capable of being used to distinguish *L. intracellularis* from related microorganisms.

Less-highly conserved regions within the *flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM*, or *ytfN* genes are particularly useful as species-specific probes and/or primers for the detection of *L. intracellularis* and very closely related species.

Furthermore, the diagnostic assays described herein can be adapted to a genus-specific or species-specific assay by varying the stringency of the hybridisation step. Accordingly, a low stringency hybridisation can be used to detect several different species of *Lawsonia* in one or more biological samples being assayed, while a high stringency hybridisation can be used to distinguish *L. intracellularis* from such other species.

The detection means according to this aspect of the invention may be any nucleic acid-based detection means such as, for example, nucleic acid hybridisation techniques or paper chromatography hybridisation assay (PACHA), or an amplification reaction such as PCR, or nucleic acid sequence-based amplification (NASBA) system. The invention further encompasses the use of different assay formats of said nucleic acid-based detection means, including restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), single-strand chain polymorphism (SSCP), amplification and mismatch detection (AMD), interspersed repetitive sequence polymerase chain reaction (IRS-PCR), inverse polymerase chain reaction (iPCR), *in situ* polymerase chain reaction and reverse transcription polymerase chain reaction (RT-PCR), amongst others.

Where the detection means is a nucleic acid hybridisation technique, the probe can be labelled with a reporter molecule capable of producing an identifiable signal (e.g., a radioisotope such as ^{32}P or ^{35}S, or a biotinylated molecule). According to this embodiment, those skilled in the art will be aware that the detection of said reporter molecule provides for identification of the probe and that, following the hybridisation reaction, the detection of the corresponding nucleotide sequences in the biological sample is facilitated. Additional probes can be used to confirm the assay results obtained using a single probe.

A variation of the nucleic acid hybridisation technique contemplated by the present invention is the paper chromatography hybridisation assay (PACHA) described by Reinhartz *et al.* (1993) and equivalents thereof, wherein a target nucleic acid molecule is labelled with a reporter molecule such as biotin, applied to one end of a nitrocellulose or nylon membrane filter strip and subjected to chromatography under the action of capillary or other forces (e.g., an electric field) for a time and under conditions sufficient to promote migration of said target nucleic acid along the length of said membrane to a zone at which a DNA probe is immobilised thereto such as, for example, in the middle region. According to this detection format, labelled target nucleic acid comprising the *Lawsonia spp.* nucleotide sequences complementary to the probe will hybridise thereto and become immobilised in that region of the membrane to which the probe is bound. Non-complementary sequences to the probe will diffuse past the site at which the probe is bound. The target nucleic acid may comprise a crude or partially-pure extract of DNA or RNA or, alternatively, an amplified or purified DNA. Additional variations of this detection means which utilise the nucleotide sequences described herein are clearly encompassed by the present invention.

Wherein the detection means is a RFLP, nucleic acid derived from the biological sample, in particular DNA, is digested with one or more restriction endonuclease enzymes and the digested DNA is subjected to electrophoresis, transferred to a solid support such as, for example, a nylon or nitrocellulose membrane, and hybridised to a probe optionally labelled with a reporter molecule as hereinbefore defined. According to this embodiment, a specific pattern of DNA fragments is displayed on the support, wherein said pattern is preferably specific for a particular *Lawsonia spp.*, to enable the user to distinguish between different species of the bacterium.

Wherein the detection means is an amplification reaction such as, for example, a polymerase chain reaction or a nucleic acid sequence-based amplification (NASBA) system or a variant thereof, one or more nucleic acid primer molecules of at least 15

contiguous nucleotides in length derivable from a gene selected from the group consisting of *flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM*, and *ytfN* genes is hybridised to nucleic acid derived from a biological sample, and nucleic acid copies of the FlgE-encoding genetic sequences in said sample, or a part or fragment thereof, are enzymically-amplified.

Those skilled in the art will be aware that there must be a sufficiently high percentage of nucleotide sequence identity between the primers and the sequences in the biological sample template molecule to which they hybridise (i.e., the "template molecule"). As stated previously, the stringency conditions can be selected to promote hybridisation.

Preferably, each primer is at least about 95% identical to a region of a gene selected from the group consisting of *flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM*, and *ytfN* genes in the template molecule to which it hybridises.

Those skilled in the art will also be aware that, in one format, PCR provides for the hybridisation of non-complementary primers to different strands of the template molecule, such that the hybridised primers are positioned to facilitate the 5′→ 3′ synthesis of nucleic acid in the intervening region, under the control of a thermostable DNA polymerase enzyme. As a consequence, PCR provides an advantage over other detection means in so far as the nucleotide sequence in the region between the hybridised primers may be unknown and unrelated to any known nucleotide sequence.

In an alternative embodiment, wherein the detection means is AFLP, the primers are selected such that, when nucleic acid derived from the biological sample, in particular DNA, is amplified, different length amplification products are produced from different *Lawsonia spp.* The amplification products can be subjected to electrophoresis, transferred to a solid support such as, for example, a nylon or nitrocellulose membrane, and hybridised to a probe optionally labelled with a reporter molecule as hereinbefore described. According to this embodiment, a specific pattern of amplified

DNA fragments is displayed on the support, said pattern optionally specific for a particular *Lawsonia ssp.,* to enable the user to distinguish between different species of the bacterium in much the same way as for RFLP analysis.

The technique of AMD facilitates, not only the detection of *Lawsonia spp.* DNA in a biological sample, but also the determination of nucleotide sequence variants which differ from the primers and probes used in the assay format. Wherein the detection means is AMD, the probe is end-labelled with a suitable reporter molecule and mixed with an excess of the amplified template molecule. The mixtures are subsequently denatured and allowed to renature to form nucleic acid "probe:template hybrid molecules" or "hybrids", such that any nucleotide sequence variation between the probe and the temple molecule to which it is hybridised will disrupt base-pairing in the hybrids. These regions of mismatch are sensitive to specific chemical modification using hydroxylamine (mismatched cytosine residues) or osmium tetroxide (mismatched thymidine residues), allowing subsequent cleavage of the modified site using piperidine. The cleaved nucleic acid may be analysed using denaturing polyacrylamide gel electrophoresis, followed by standard nucleic acid hybridisation as described *supra,* to detect the *Lawsonia*-derived nucleotide sequences. Those skilled in the art will be aware of the means of end-labelling a genetic probe according to the performance of the invention described in this embodiment.

According to this embodiment, the use of a single end-labelled probe allows unequivocal localisation of the sequence variation. The distance between the point(s) of sequence variation and the end-label is represented by the size of the cleavage product.

In an alternative embodiment of AMD, the probe is labelled at both ends with a reporter molecule, to facilitate the simultaneous analysis of both DNA strands.

Wherein the detection means is RT-PCR, the nucleic acid sample comprises an RNA molecule which is a transcription product of *Lawsonia*-derived DNA or a homologue,

analogue or derivative thereof. As a consequence, this assay format is particularly useful when it is desirable to determine expression of one or more *Lawsonia* genes. According to this embodiment, the RNA sample is reverse-transcribed to produce the complementary single-stranded DNA which is subsequently amplified using standard procedures.

Variations of the embodiments described herein are described in detail by McPherson *et al.* (1991).

The present invention clearly extends to the use of any and all detection means referred to *supra* for the purposes of diagnosing *Lawsonia spp.* and in particular *L. intracellularis* infection in animals.

The amplification reaction detection means described *supra* can be further coupled to a classical hybridisation reaction detection means to further enhance sensitivity and specificity of the inventive method, such as by hybridising the amplified DNA with a probe which is different from any of the primers used in the amplification reaction.

Similarly, the hybridisation reaction detection means described *supra* can be further coupled to a second hybridisation step employing a probe which is different from the probe used in the first hybridisation reaction.

A further aspect of the invention provides an isolated probe or primer derived from a gene selected from the group consisting of *flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM*, and *ytfN* genes. Preferably, the probe or primer of the invention comprises a nucleotide sequence selected from the group consisting of SEQ ID NO: 19 to SEQ ID NO: 46 or a complementary nucleotide sequence thereto.

The present invention does not extend to any nucleic acid or polypeptide of *Camplylobacter* or *Helicobacter* that was disclosed publicly before the filing date or priority date of this application, or otherwise takes priority over the instant application,

and which is homologous to a nucleotide sequence or amino acid sequence of *Lawsonia spp.* disclosed herein.

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Agriculture Victoria Services Pty Ltd AND Pig Research and Development Corporation AND Pfizer Products Inc.

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Ser Leu Lys Gly Ile Phe Glu Asp Glu Gly His Glu Val Leu Glu Arg
            20                  25                  30

gct tca gca gaa gaa gga ctt aag tgt gtt gat gta gag tct cca gat   144
Ala Ser Ala Glu Glu Gly Leu Lys Cys Val Asp Val Glu Ser Pro Asp
        35                  40                  45

ctt gtt ttt ctt gat att tgg ctt cct ggg atg gat ggt ctt atg gct   192
Leu Val Phe Leu Asp Ile Trp Leu Pro Gly Met Asp Gly Leu Met Ala
    50                  55                  60

tta gac cat att cag gct ctt cat cag gaa tta cct gtt att atg att   240
Leu Asp His Ile Gln Ala Leu His Gln Glu Leu Pro Val Ile Met Ile
65                  70                  75                  80

tca ggt cat gcc aca att gaa act gct gta aca gct atc cgt caa ggt   288
Ser Gly His Ala Thr Ile Glu Thr Ala Val Thr Ala Ile Arg Gln Gly
                85                  90                  95

gct tat gat ttt att gaa aag cct ctt tct ttg gaa aaa gtc ctt att   336
Ala Tyr Asp Phe Ile Glu Lys Pro Leu Ser Leu Glu Lys Val Leu Ile
            100                 105                 110

aca gct aat aga gct ata gaa aca gta aga tta aga agg gaa aac aaa   384
Thr Ala Asn Arg Ala Ile Glu Thr Val Arg Leu Arg Arg Glu Asn Lys
        115                 120                 125

tta cta cgt act gta tta cct gag gag agt gag ttt ata gga cag tct   432
Leu Leu Arg Thr Val Leu Pro Glu Glu Ser Glu Phe Ile Gly Gln Ser
    130                 135                 140

cct gtt atc tta aaa ttt aaa agt tta tta tca cag gtc gct cca aca   480
Pro Val Ile Leu Lys Phe Lys Ser Leu Leu Ser Gln Val Ala Pro Thr
```

145 150 155 160

gat gct tgg gta cta ctt aca gga gag aat ggt aca ggt aaa gag tta 528
Asp Ala Trp Val Leu Leu Thr Gly Glu Asn Gly Thr Gly Lys Glu Leu
165 170 175

gct gca caa gca ttg cac aaa gga agc tca cga tat caa aaa cca ttt 576
Ala Ala Gln Ala Leu His Lys Gly Ser Ser Arg Tyr Gln Lys Pro Phe
180 185 190

ata gct gtt aat tgt gct gct atc cct gaa gaa ttg att gaa agc gaa 624
Ile Ala Val Asn Cys Ala Ala Ile Pro Glu Glu Leu Ile Glu Ser Glu
195 200 205

cta ttt ggt cat gaa aaa ggg gcc ttt act ggt gcc gat gct tct cgt 672
Leu Phe Gly His Glu Lys Gly Ala Phe Thr Gly Ala Asp Ala Ser Arg
210 215 220

gca ggt cgt ttt gag ttg gca cat aaa gga aca tta ttt ctt gat gaa 720
Ala Gly Arg Phe Glu Leu Ala His Lys Gly Thr Leu Phe Leu Asp Glu
225 230 235 240

ata gga gat atg agt tta aaa aca caa gca aaa att ttg cgt att ttg 768
Ile Gly Asp Met Ser Leu Lys Thr Gln Ala Lys Ile Leu Arg Ile Leu
245 250 255

caa gaa caa tgt ttt gaa aaa att ggt agt gtt aga act att aaa gtt 816
Gln Glu Gln Cys Phe Glu Lys Ile Gly Ser Val Arg Thr Ile Lys Val
260 265 270

gat gta aga gtt att gca gca aca aat aag aat ctt gaa gac gct att 864
Asp Val Arg Val Ile Ala Ala Thr Asn Lys Asn Leu Glu Asp Ala Ile
275 280 285

agc gat gga aca ttt cgt caa gat ttg tat tat cgc tta cga gtt gtt 912
Ser Asp Gly Thr Phe Arg Gln Asp Leu Tyr Tyr Arg Leu Arg Val Val
290 295 300

cca ttg cat ctt ccc cct ctt cgt gaa cgt gat tct gat att gag cta 960
Pro Leu His Leu Pro Pro Leu Arg Glu Arg Asp Ser Asp Ile Glu Leu
305 310 315 320

tta tta aat agg ttt gtg att cag ttg agt aaa cgt tat aga cgt gag 1008
Leu Leu Asn Arg Phe Val Ile Gln Leu Ser Lys Arg Tyr Arg Arg Glu
325 330 335

```
ccg cct att ttt tta gat gag gtc ttc cct gta ttg aaa caa tat tgt   1056
Pro Pro Ile Phe Leu Asp Glu Val Phe Pro Val Leu Lys Gln Tyr Cys
            340                 345                 350

tgg cca ggg aat gta aga gaa tta ctt aat ttt gta gaa cga atg gtt   1104
Trp Pro Gly Asn Val Arg Glu Leu Leu Asn Phe Val Glu Arg Met Val
        355                 360                 365

att ctt tat tca ggg aag aaa gta tgt ttg aca gat cct aag gta aaa   1152
Ile Leu Tyr Ser Gly Lys Lys Val Cys Leu Thr Asp Pro Lys Val Lys
    370                 375                 380

agc aat tta aaa tat tta ccc aag aaa ttt tct tcc cat tat aac ttt   1200
Ser Asn Leu Lys Tyr Leu Pro Lys Lys Phe Ser Ser His Tyr Asn Phe
385                 390                 395                 400

ctt ccc gat ata gat ttt aac cag gct aaa ata gct ttt gaa cca aaa   1248
Leu Pro Asp Ile Asp Phe Asn Gln Ala Lys Ile Ala Phe Glu Pro Lys
                405                 410                 415

ttt tta act gaa aaa tta cat gct tat caa gga aat att acc cga tta   1296
Phe Leu Thr Glu Lys Leu His Ala Tyr Gln Gly Asn Ile Thr Arg Leu
            420                 425                 430

gca gaa gct att gga ctt gaa aga agt tat tta tat aga aag cta aaa   1344
Ala Glu Ala Ile Gly Leu Glu Arg Ser Tyr Leu Tyr Arg Lys Leu Lys
        435                 440                 445

agc tat ggt att tat ctg tct gag tga                              1371
Ser Tyr Gly Ile Tyr Leu Ser Glu
    450                 455
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<210> 6
<211> 456
<212> PRT
<213> Lawsonia intracellularis

<400> 6

```
Met Ser Ala Arg Ile Leu Ile Ile Asp Asp Glu Asp Ser Ile Arg Phe
  1               5                  10                  15

Ser Leu Lys Gly Ile Phe Glu Asp Glu Gly His Glu Val Leu Glu Arg
            20                  25                  30
```

```
Ala Ser Ala Glu Glu Gly Leu Lys Cys Val Asp Val Glu Ser Pro Asp
          35                  40                  45

Leu Val Phe Leu Asp Ile Trp Leu Pro Gly Met Asp Gly Leu Met Ala
     50                  55                  60

Leu Asp His Ile Gln Ala Leu His Gln Glu Leu Pro Val Ile Met Ile
 65                  70                  75                  80

Ser Gly His Ala Thr Ile Glu Thr Ala Val Thr Ala Ile Arg Gln Gly
                 85                  90                  95

Ala Tyr Asp Phe Ile Glu Lys Pro Leu Ser Leu Glu Lys Val Leu Ile
            100                 105                 110

Thr Ala Asn Arg Ala Ile Glu Thr Val Arg Leu Arg Arg Glu Asn Lys
        115                 120                 125

Leu Leu Arg Thr Val Leu Pro Glu Glu Ser Glu Phe Ile Gly Gln Ser
    130                 135                 140

Pro Val Ile Leu Lys Phe Lys Ser Leu Leu Ser Gln Val Ala Pro Thr
145                 150                 155                 160

Asp Ala Trp Val Leu Leu Thr Gly Glu Asn Gly Thr Gly Lys Glu Leu
                165                 170                 175

Ala Ala Gln Ala Leu His Lys Gly Ser Ser Arg Tyr Gln Lys Pro Phe
            180                 185                 190

Ile Ala Val Asn Cys Ala Ala Ile Pro Glu Glu Leu Ile Glu Ser Glu
        195                 200                 205

Leu Phe Gly His Glu Lys Gly Ala Phe Thr Gly Ala Asp Ala Ser Arg
    210                 215                 220

Ala Gly Arg Phe Glu Leu Ala His Lys Gly Thr Leu Phe Leu Asp Glu
225                 230                 235                 240

Ile Gly Asp Met Ser Leu Lys Thr Gln Ala Lys Ile Leu Arg Ile Leu
                245                 250                 255

Gln Glu Gln Cys Phe Glu Lys Ile Gly Ser Val Arg Thr Ile Lys Val
            260                 265                 270
```

```
Asp Val Arg Val Ile Ala Ala Thr Asn Lys Asn Leu Glu Asp Ala Ile
        275                 280                 285

Ser Asp Gly Thr Phe Arg Gln Asp Leu Tyr Tyr Arg Leu Arg Val Val
    290                 295                 300

Pro Leu His Leu Pro Pro Leu Arg Glu Arg Asp Ser Asp Ile Glu Leu
305                 310                 315                 320

Leu Leu Asn Arg Phe Val Ile Gln Leu Ser Lys Arg Tyr Arg Arg Glu
                325                 330                 335

Pro Pro Ile Phe Leu Asp Glu Val Phe Pro Val Leu Lys Gln Tyr Cys
            340                 345                 350

Trp Pro Gly Asn Val Arg Glu Leu Leu Asn Phe Val Glu Arg Met Val
        355                 360                 365

Ile Leu Tyr Ser Gly Lys Lys Val Cys Leu Thr Asp Pro Lys Val Lys
    370                 375                 380

Ser Asn Leu Lys Tyr Leu Pro Lys Lys Phe Ser Ser His Tyr Asn Phe
385                 390                 395                 400

Leu Pro Asp Ile Asp Phe Asn Gln Ala Lys Ile Ala Phe Glu Pro Lys
                405                 410                 415

Phe Leu Thr Glu Lys Leu His Ala Tyr Gln Gly Asn Ile Thr Arg Leu
            420                 425                 430

Ala Glu Ala Ile Gly Leu Glu Arg Ser Tyr Leu Tyr Arg Lys Leu Lys
        435                 440                 445

Ser Tyr Gly Ile Tyr Leu Ser Glu
    450                 455

<210> 7
<211> 412
<212> DNA
<213> Lawsonia intracellularis

<220>
<221> CDS
<222> (1)..(411)
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<400> 7

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aaa caa att gat ata atc att agt ggg gct acg ata act ctt gaa cgt   48
Lys Gln Ile Asp Ile Ile Ile Ser Gly Ala Thr Ile Thr Leu Glu Arg
 1               5                  10                  15

aat ctt caa gtc aat ttt tct aac cca tac cat caa aca gat att gaa   96
Asn Leu Gln Val Asn Phe Ser Asn Pro Tyr His Gln Thr Asp Ile Glu
            20                  25                  30

gtc ctg gct aat gca aaa aaa gtt aaa ggg atg aag ttt cca caa gac   144
Val Leu Ala Asn Ala Lys Lys Val Lys Gly Met Lys Phe Pro Gln Asp
        35                  40                  45

ttt aat aaa cct gaa gtt ata gtt gct ata cgt aat ggt agt aca gtt   192
Phe Asn Lys Pro Glu Val Ile Val Ala Ile Arg Asn Gly Ser Thr Val
    50                  55                  60

att act cct gca aag caa ctt ctt cct aaa gca tct ttt aga ctc ttt   240
Ile Thr Pro Ala Lys Gln Leu Leu Pro Lys Ala Ser Phe Arg Leu Phe
65                  70                  75                  80

gat gat gaa gtt gca tct ata aaa gat gta gaa tct gga caa tca cat   288
Asp Asp Glu Val Ala Ser Ile Lys Asp Val Glu Ser Gly Gln Ser His
                85                  90                  95

ata tta tta gct tca gca cca tta cca gcg att caa gct ata aac tca   336
Ile Leu Leu Ala Ser Ala Pro Leu Pro Ala Ile Gln Ala Ile Asn Ser
            100                 105                 110

aat ggc aac ctt att cgt tta gat aca ctc ccc att act cat caa tct   384
Asn Gly Asn Leu Ile Arg Leu Asp Thr Leu Pro Ile Thr His Gln Ser
        115                 120                 125

gta gga ttt gca ata aag aag gga gat c                            412
Val Gly Phe Ala Ile Lys Lys Gly Asp
    130                 135
```

<210> 8
<211> 137
<212> PRT
<213> Lawsonia intracellularis

<400> 8

```
Lys Gln Ile Asp Ile Ile Ile Val Gly Ala Thr Ile Thr Leu Glu Arg
 1               5                  10                  15

Asn Leu Gln Val Asn Phe Ser Asn Pro Tyr His Gln Thr Asp Ile Glu
            20                  25                  30

Val Leu Ala Asn Ala Lys Lys Val Lys Gly Met Lys Phe Pro Gln Asp
        35                  40                  45

Phe Asn Lys Pro Glu Val Ile Val Ala Ile Arg Asn Gly Ser Thr Val
    50                  55                  60

Ile Thr Pro Ala Lys Gln Leu Leu Pro Lys Ala Ser Phe Arg Leu Phe
65                  70                  75                  80

Asp Asp Glu Val Ala Ser Ile Lys Asp Val Glu Ser Gly Gln Ser His
                85                  90                  95

Ile Leu Leu Ala Ser Ala Pro Leu Pro Ala Ile Gln Ala Ile Asn Ser
            100                 105                 110

Asn Gly Asn Leu Ile Arg Leu Asp Thr Leu Pro Ile Thr His Gln Ser
        115                 120                 125

Val Gly Phe Ala Ile Lys Lys Gly Asp
    130                 135
```

<210> 9
<211> 849
<212> DNA
<213> Lawsonia intracellularis

<220>
<221> CDS
<222> (1)..(846)

<400> 9

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atg tat att att att gga tac ttt att gtt att gct tcc att att gga   48
Met Tyr Ile Ile Ile Gly Tyr Phe Ile Val Ile Ala Ser Ile Ile Gly
 1               5                  10                  15

ggc tac ctt atg gct aaa ggg aat ctt gct tta ctc ttt caa cct gca   96
Gly Tyr Leu Met Ala Lys Gly Asn Leu Ala Leu Leu Phe Gln Pro Ala
            20                  25                  30
```

```
gaa ctt gtt atc att att ggg gca gca tta ggt gct ttt ttt gct tca   144
Glu Leu Val Ile Ile Ile Gly Ala Ala Leu Gly Ala Phe Phe Ala Ser
          35                  40                  45

cag acg aaa tat tca ttt act ctg gtc att aaa aat tta tca cac att   192
Gln Thr Lys Tyr Ser Phe Thr Leu Val Ile Lys Asn Leu Ser His Ile
      50                  55                  60

ttt ggc gat cca aac agt aca aaa ata aaa tac ctt gaa aca ctt gcc   240
Phe Gly Asp Pro Asn Ser Thr Lys Ile Lys Tyr Leu Glu Thr Leu Ala
 65                  70                  75                  80

ctt ctc tat gga ctt ttc tta aaa atg aat aga gaa ggt gtc att agt   288
Leu Leu Tyr Gly Leu Phe Leu Lys Met Asn Arg Glu Gly Val Ile Ser
                 85                  90                  95

ata gaa agt gat ata gaa aaa cct gaa tca agt cct atc ttt agt aaa   336
Ile Glu Ser Asp Ile Glu Lys Pro Glu Ser Ser Pro Ile Phe Ser Lys
            100                 105                 110

tac cct aca att gta aaa gat act aaa gtt gtt gcc ttt att gca gat   384
Tyr Pro Thr Ile Val Lys Asp Thr Lys Val Val Ala Phe Ile Ala Asp
        115                 120                 125

aca tta cga gtt tat ctg aca aca ggt gca cca gaa gat ata gat aac   432
Thr Leu Arg Val Tyr Leu Thr Thr Gly Ala Pro Glu Asp Ile Asp Asn
    130                 135                 140

ctc atg gaa tct gac atg aaa att aca cac gaa gaa gaa tta tta cct   480
Leu Met Glu Ser Asp Met Lys Ile Thr His Glu Glu Glu Leu Leu Pro
145                 150                 155                 160

gca cat tcc atc agc cat atg gca gag tcg cta cca gga atg ggt att   528
Ala His Ser Ile Ser His Met Ala Glu Ser Leu Pro Gly Met Gly Ile
                165                 170                 175

gtt gct gca gta tta ggt gtt gtt att acc atg gga aaa att aat gag   576
Val Ala Ala Val Leu Gly Val Val Ile Thr Met Gly Lys Ile Asn Glu
            180                 185                 190

cct cca gaa gtc ctt ggg cat tat att gga gca gct ttg gtt ggt aca   624
Pro Pro Glu Val Leu Gly His Tyr Ile Gly Ala Ala Leu Val Gly Thr
        195                 200                 205
```

```
ttt ata ggt att ctt ttc tgt tat ggt ttt ttt gga cct atg ggt tca   672
Phe Ile Gly Ile Leu Phe Cys Tyr Gly Phe Phe Gly Pro Met Gly Ser
    210                 215                 220

aag ctt gaa acc tct gca gaa gaa gca cat ttt tat tat aat tcc att   720
Lys Leu Glu Thr Ser Ala Glu Glu Ala His Phe Tyr Tyr Asn Ser Ile
225                 230                 235                 240

aaa gaa gct gtt gca gct gct atc cga ggt tct aca cca atg ata gca   768
Lys Glu Ala Val Ala Ala Ala Ile Arg Gly Ser Thr Pro Met Ile Ala
                245                 250                 255

gta gaa tat gga aga cgt gcc ata cct aat aca ttt cgt cca tca ttt   816
Val Glu Tyr Gly Arg Arg Ala Ile Pro Asn Thr Phe Arg Pro Ser Phe
            260                 265                 270

tcg gaa atg gaa gaa cgt cta aaa aca gga taa                      849
Ser Glu Met Glu Glu Arg Leu Lys Thr Gly
        275                 280
```

<210> 10
<211> 282
<212> PRT
<213> Lawsonia intracellularis

<400> 10

```
Met Tyr Ile Ile Ile Gly Tyr Phe Ile Val Ile Ala Ser Ile Ile Gly
  1               5                  10                  15

Gly Tyr Leu Met Ala Lys Gly Asn Leu Ala Leu Leu Phe Gln Pro Ala
            20                  25                  30

Glu Leu Val Ile Ile Ile Gly Ala Ala Leu Gly Ala Phe Phe Ala Ser
        35                  40                  45

Gln Thr Lys Tyr Ser Phe Thr Leu Val Ile Lys Asn Leu Ser His Ile
    50                  55                  60

Phe Gly Asp Pro Asn Ser Thr Lys Ile Lys Tyr Leu Glu Thr Leu Ala
65                  70                  75                  80

Leu Leu Tyr Gly Leu Phe Leu Lys Met Asn Arg Glu Gly Val Ile Ser
                85                  90                  95
```

Ile Glu Ser Asp Ile Glu Lys Pro Glu Ser Ser Pro Ile Phe Ser Lys
100 105 110

Tyr Pro Thr Ile Val Lys Asp Thr Lys Val Val Ala Phe Ile Ala Asp
115 120 125

Thr Leu Arg Val Tyr Leu Thr Thr Gly Ala Pro Glu Asp Ile Asp Asn
130 135 140

Leu Met Glu Ser Asp Met Lys Ile Thr His Glu Glu Glu Leu Leu Pro
145 150 155 160

Ala His Ser Ile Ser His Met Ala Glu Ser Leu Pro Gly Met Gly Ile
165 170 175

Val Ala Ala Val Leu Gly Val Val Ile Thr Met Gly Lys Ile Asn Glu
180 185 190

Pro Pro Glu Val Leu Gly His Tyr Ile Gly Ala Ala Leu Val Gly Thr
195 200 205

Phe Ile Gly Ile Leu Phe Cys Tyr Gly Phe Phe Gly Pro Met Gly Ser
210 215 220

Lys Leu Glu Thr Ser Ala Glu Glu Ala His Phe Tyr Tyr Asn Ser Ile
225 230 235 240

Lys Glu Ala Val Ala Ala Ala Ile Arg Gly Ser Thr Pro Met Ile Ala
245 250 255

Val Glu Tyr Gly Arg Arg Ala Ile Pro Asn Thr Phe Arg Pro Ser Phe
260 265 270

Ser Glu Met Glu Glu Arg Leu Lys Thr Gly
275 280

<210> 11
<211> 717
<212> DNA
<213> Lawsonia intracellularis

<220>
<221> CDS
<222> (1)..(714)

<400> 11

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atg tct ggc tca tgg aaa gtg gct tat gca gac ttt gtt aca gct atg   48
Met Ser Gly Ser Trp Lys Val Ala Tyr Ala Asp Phe Val Thr Ala Met
 1               5                  10                  15

atg gct ttc ttt cta ctg atg tgg att ctt gca atg aca ccc cct gag   96
Met Ala Phe Phe Leu Leu Met Trp Ile Leu Ala Met Thr Pro Pro Glu
            20                  25                  30

gtt aaa gaa ggt ctt gct gca tat ttt tct tca tct gat gct aca ttt   144
Val Lys Glu Gly Leu Ala Ala Tyr Phe Ser Ser Ser Asp Ala Thr Phe
        35                  40                  45

aaa aca cct gat agt tcg cca atc tct aac aat cct ctt atc aac caa   192
Lys Thr Pro Asp Ser Ser Pro Ile Ser Asn Asn Pro Leu Ile Asn Gln
    50                  55                  60

ata gat aaa ctt gat act cga caa tta aaa att aat gaa aca gaa caa   240
Ile Asp Lys Leu Asp Thr Arg Gln Leu Lys Ile Asn Glu Thr Glu Gln
65                  70                  75                  80

tct cat tat gct ctt gct aat aaa tta aaa aaa atg tta atg gct gat   288
Ser His Tyr Ala Leu Ala Asn Lys Leu Lys Lys Met Leu Met Ala Asp
                85                  90                  95

gct atc cca cag tca gca aca gga ata agt gct gac gat gtt ggt gta   336
Ala Ile Pro Gln Ser Ala Thr Gly Ile Ser Ala Asp Asp Val Gly Val
            100                 105                 110

tta tta cgt gta aat tct aat tcc acg ttt ttt cct ggt aca gca act   384
Leu Leu Arg Val Asn Ser Asn Ser Thr Phe Phe Pro Gly Thr Ala Thr
        115                 120                 125

ctt aca ccc gaa ggg aaa aaa gtt atg gga act gtt tta gcc gtt ctc   432
Leu Thr Pro Glu Gly Lys Lys Val Met Gly Thr Val Leu Ala Val Leu
    130                 135                 140

cgt gaa tat aat ctt tac ctt gtg ata cgt ggc cat gct gat att ggt   480
Arg Glu Tyr Asn Leu Tyr Leu Val Ile Arg Gly His Ala Asp Ile Gly
145                 150                 155                 160

gaa ata aca aaa ggc agc cct ttt gct tct aac tgg gaa ctt tca gga   528
Glu Ile Thr Lys Gly Ser Pro Phe Ala Ser Asn Trp Glu Leu Ser Gly
                165                 170                 175
```

```
gct cgt gca gct gca gct gca cag tat ctt gta gag cac ggg ata aag   576
Ala Arg Ala Ala Ala Ala Ala Gln Tyr Leu Val Glu His Gly Ile Lys
            180                 185                 190

gct tca cga att cgc tct gta gga tat gca gat aca aga cct cta gaa   624
Ala Ser Arg Ile Arg Ser Val Gly Tyr Ala Asp Thr Arg Pro Leu Glu
        195                 200                 205

cct agt tct cct gaa gga agt aca aaa aat cgt cgt ata gaa ttc tat   672
Pro Ser Ser Pro Glu Gly Ser Thr Lys Asn Arg Arg Ile Glu Phe Tyr
    210                 215                 220

ttt cat cgg cca gaa gtt atg tct tat ggc gtt gta tat taa tag       717
Phe His Arg Pro Glu Val Met Ser Tyr Gly Val Val Tyr
225                 230                 235
```

<210> 12
<211> 237
<212> PRT
<213> Lawsonia intracellularis

<400> 12

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Met Ser Gly Ser Trp Lys Val Ala Tyr Ala Asp Phe Val Thr Ala Met
  1               5                  10                  15

Met Ala Phe Phe Leu Leu Met Trp Ile Leu Ala Met Thr Pro Pro Glu
            20                  25                  30

Val Lys Glu Gly Leu Ala Ala Tyr Phe Ser Ser Ser Asp Ala Thr Phe
        35                  40                  45

Lys Thr Pro Asp Ser Ser Pro Ile Ser Asn Asn Pro Leu Ile Asn Gln
    50                  55                  60

Ile Asp Lys Leu Asp Thr Arg Gln Leu Lys Ile Asn Glu Thr Glu Gln
65                  70                  75                  80

Ser His Tyr Ala Leu Ala Asn Lys Leu Lys Lys Met Leu Met Ala Asp
                85                  90                  95

Ala Ile Pro Gln Ser Ala Thr Gly Ile Ser Ala Asp Asp Val Gly Val
            100                 105                 110
```

```
Leu Leu Arg Val Asn Ser Asn Ser Thr Phe Phe Pro Gly Thr Ala Thr
        115                 120                 125

Leu Thr Pro Glu Gly Lys Lys Val Met Gly Thr Val Leu Ala Val Leu
    130                 135                 140

Arg Glu Tyr Asn Leu Tyr Leu Val Ile Arg Gly His Ala Asp Ile Gly
145                 150                 155                 160

Glu Ile Thr Lys Gly Ser Pro Phe Ala Ser Asn Trp Glu Leu Ser Gly
                165                 170                 175

Ala Arg Ala Ala Ala Ala Ala Gln Tyr Leu Val Glu His Gly Ile Lys
            180                 185                 190

Ala Ser Arg Ile Arg Ser Val Gly Tyr Ala Asp Thr Arg Pro Leu Glu
        195                 200                 205

Pro Ser Ser Pro Glu Gly Ser Thr Lys Asn Arg Arg Ile Glu Phe Tyr
    210                 215                 220

Phe His Arg Pro Glu Val Met Ser Tyr Gly Val Val Tyr
225                 230                 235
```

<210> 13
<211> 1047
<212> DNA
<213> Lawsonia intracellularis

<220>
<221> CDS
<222> (1)..(1044)

<400> 13

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atg ata atc ctt tta gga act gtt ttt ctt att gtt ctt atc tct gca   48
Met Ile Ile Leu Leu Gly Thr Val Phe Leu Ile Val Leu Ile Ser Ala
  1               5                  10                  15

tta tgc tca atg atg gaa gct gct ata tac tct atc cct att act tat   96
Leu Cys Ser Met Met Glu Ala Ala Ile Tyr Ser Ile Pro Ile Thr Tyr
             20                  25                  30

att gaa cac ctt cgt gaa cag gga agc aaa aaa gga gaa aaa ctt tat   144
Ile Glu His Leu Arg Glu Gln Gly Ser Lys Lys Gly Glu Lys Leu Tyr
```

```
          35                  40                  45

tat tta cat agt aat att gat cag cct att aca gcc gta tta ata ttg   192
Tyr Leu His Ser Asn Ile Asp Gln Pro Ile Thr Ala Val Leu Ile Leu
     50                  55                  60

aat act ata gca aat act gct gga gct gcc ctt gct gga gca att gct   240
Asn Thr Ile Ala Asn Thr Ala Gly Ala Ala Leu Ala Gly Ala Ile Ala
 65                  70                  75                  80

aca aca aca ctt cat gaa tct act aag cct ttc ttt gca gca atc ctc   288
Thr Thr Thr Leu His Glu Ser Thr Lys Pro Phe Phe Ala Ala Ile Leu
                 85                  90                  95

acc ttg ctt att tta gct ttt ggg gaa att ata cct aaa aca cta ggt   336
Thr Leu Leu Ile Leu Ala Phe Gly Glu Ile Ile Pro Lys Thr Leu Gly
            100                 105                 110

gtt gct tac tct aaa cgt att gct ata att ctc ctt aat cct ctc tct   384
Val Ala Tyr Ser Lys Arg Ile Ala Ile Ile Leu Leu Asn Pro Leu Ser
        115                 120                 125

att ctt ata gtt act tta aaa ccc ctt att atg ctt tca agc tac tta   432
Ile Leu Ile Val Thr Leu Lys Pro Leu Ile Met Leu Ser Ser Tyr Leu
    130                 135                 140

aca cga ctt gtt tca cct cga aaa cgt cct aca gtt aca gaa gat gac   480
Thr Arg Leu Val Ser Pro Arg Lys Arg Pro Thr Val Thr Glu Asp Asp
145                 150                 155                 160

atc cgt gca ctt aca agt ctt tcc aga gag tct ggt cgt att aag cca   528
Ile Arg Ala Leu Thr Ser Leu Ser Arg Glu Ser Gly Arg Ile Lys Pro
                165                 170                 175

tat gaa gaa cat gtc ata aaa aat atc ctt agt ctt gat tta aaa tat   576
Tyr Glu Glu His Val Ile Lys Asn Ile Leu Ser Leu Asp Leu Lys Tyr
            180                 185                 190

gct cat gaa att atg act ccc aga act atg gtc ttt tca ctt cat gaa   624
Ala His Glu Ile Met Thr Pro Arg Thr Met Val Phe Ser Leu His Glu
        195                 200                 205

aac ctt act gtc tct gaa gct tat agc aac ccc aaa ata tgg aac tat   672
Asn Leu Thr Val Ser Glu Ala Tyr Ser Asn Pro Lys Ile Trp Asn Tyr
    210                 215                 220
```

```
agt cgc atc cct act tat gga gaa aat aac gaa gac att act ggc att   720
Ser Arg Ile Pro Thr Tyr Gly Glu Asn Asn Glu Asp Ile Thr Gly Ile
225                 230                 235                 240

atc caa cga tat gaa att gga cga tat atg acc aat gga gaa aca gaa   768
Ile Gln Arg Tyr Glu Ile Gly Arg Tyr Met Thr Asn Gly Glu Thr Glu
                245                 250                 255

aaa aaa ctt tta gaa att atg caa cca gca aaa ttt gtc ctt gaa agt   816
Lys Lys Leu Leu Glu Ile Met Gln Pro Ala Lys Phe Val Leu Glu Ser
            260                 265                 270

caa act gta gat cat tta ctt ctt gca ttt tta gaa gaa aga caa cat   864
Gln Thr Val Asp His Leu Leu Leu Ala Phe Leu Glu Glu Arg Gln His
        275                 280                 285

ctt ttt att gta ctt gat gag tat ggg gga tta tct ggt gtt gtt tcc   912
Leu Phe Ile Val Leu Asp Glu Tyr Gly Gly Leu Ser Gly Val Val Ser
    290                 295                 300

tta gaa gat gta tta gaa act atg ctt gga aga gaa att gtt gat gaa   960
Leu Glu Asp Val Leu Glu Thr Met Leu Gly Arg Glu Ile Val Asp Glu
305                 310                 315                 320

agt gat aca aca cct gat ctt aga gca ctt gca aaa aaa aga cat agt   1008
Ser Asp Thr Thr Pro Asp Leu Arg Ala Leu Ala Lys Lys Arg His Ser
                325                 330                 335

gca tta atc caa aat aat aaa aat act ctt tta aaa taa               1047
Ala Leu Ile Gln Asn Asn Lys Asn Thr Leu Leu Lys
            340                 345
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<210> 14
<211> 348
<212> PRT
<213> Lawsonia intracellularis

<400> 14

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Met Ile Ile Leu Leu Gly Thr Val Phe Leu Ile Val Leu Ile Ser Ala
  1               5                  10                  15

Leu Cys Ser Met Met Glu Ala Ala Ile Tyr Ser Ile Pro Ile Thr Tyr
             20                  25                  30
```

Ile Glu His Leu Arg Glu Gln Gly Ser Lys Lys Gly Glu Lys Leu Tyr
35 40 45

Tyr Leu His Ser Asn Ile Asp Gln Pro Ile Thr Ala Val Leu Ile Leu
50 55 60

Asn Thr Ile Ala Asn Thr Ala Gly Ala Ala Leu Ala Gly Ala Ile Ala
65 70 75 80

Thr Thr Thr Leu His Glu Ser Thr Lys Pro Phe Phe Ala Ala Ile Leu
85 90 95

Thr Leu Leu Ile Leu Ala Phe Gly Glu Ile Ile Pro Lys Thr Leu Gly
100 105 110

Val Ala Tyr Ser Lys Arg Ile Ala Ile Ile Leu Leu Asn Pro Leu Ser
115 120 125

Ile Leu Ile Val Thr Leu Lys Pro Leu Ile Met Leu Ser Ser Tyr Leu
130 135 140

Thr Arg Leu Val Ser Pro Arg Lys Arg Pro Thr Val Thr Glu Asp Asp
145 150 155 160

Ile Arg Ala Leu Thr Ser Leu Ser Arg Glu Ser Gly Arg Ile Lys Pro
165 170 175

Tyr Glu Glu His Val Ile Lys Asn Ile Leu Ser Leu Asp Leu Lys Tyr
180 185 190

Ala His Glu Ile Met Thr Pro Arg Thr Met Val Phe Ser Leu His Glu
195 200 205

Asn Leu Thr Val Ser Glu Ala Tyr Ser Asn Pro Lys Ile Trp Asn Tyr
210 215 220

Ser Arg Ile Pro Thr Tyr Gly Glu Asn Asn Glu Asp Ile Thr Gly Ile
225 230 235 240

Ile Gln Arg Tyr Glu Ile Gly Arg Tyr Met Thr Asn Gly Glu Thr Glu
245 250 255

Lys Lys Leu Leu Glu Ile Met Gln Pro Ala Lys Phe Val Leu Glu Ser
260 265 270

```
Gln Thr Val Asp His Leu Leu Leu Ala Phe Leu Glu Glu Arg Gln His
        275                 280                 285

Leu Phe Ile Val Leu Asp Glu Tyr Gly Gly Leu Ser Gly Val Val Ser
    290                 295                 300

Leu Glu Asp Val Leu Glu Thr Met Leu Gly Arg Glu Ile Val Asp Glu
305                 310                 315                 320

Ser Asp Thr Thr Pro Asp Leu Arg Ala Leu Ala Lys Lys Arg His Ser
                325                 330                 335

Ala Leu Ile Gln Asn Asn Lys Asn Thr Leu Leu Lys
            340                 345
```

<210> 15
<211> 1812
<212> DNA
<213> Lawsonia intracellularis

<220>
<221> CDS
<222> (1)..(1809)

<400> 15

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atg caa aaa gta tgt tat ttt ttt ctt ata acc ttt ttc tac ttt ttc   48
Met Gln Lys Val Cys Tyr Phe Phe Leu Ile Thr Phe Phe Tyr Phe Phe
  1               5                  10                  15

ata aca gaa aat tat ctc ttt gct aca tca att acc act tcc aca att   96
Ile Thr Glu Asn Tyr Leu Phe Ala Thr Ser Ile Thr Thr Ser Thr Ile
             20                  25                  30

aac caa caa cat ata gca tat aca gtt act ttt acc tct cca gaa aat   144
Asn Gln Gln His Ile Ala Tyr Thr Val Thr Phe Thr Ser Pro Glu Asn
         35                  40                  45

cct aat ctt gca aca gag atg gaa aca cat agt gaa tta gta aag ctt   192
Pro Asn Leu Ala Thr Glu Met Glu Thr His Ser Glu Leu Val Lys Leu
     50                  55                  60

gca aat caa tct tta gat agt aaa ata ggt tta aat tta cgt gtt aaa   240
Ala Asn Gln Ser Leu Asp Ser Lys Ile Gly Leu Asn Leu Arg Val Lys
```

65 70 75 80

gaa gat ata agt aca gca caa aaa att ctt gac tcg aat ggt tat tat 288
Glu Asp Ile Ser Thr Ala Gln Lys Ile Leu Asp Ser Asn Gly Tyr Tyr
85 90 95

agt gga agt gtc gag gga aag att gac tgg cag acg aac cct att agt 336
Ser Gly Ser Val Glu Gly Lys Ile Asp Trp Gln Thr Asn Pro Ile Ser
100 105 110

atc caa atc caa ttt aaa cca aat gta caa tat aaa ata aat aca ata 384
Ile Gln Ile Gln Phe Lys Pro Asn Val Gln Tyr Lys Ile Asn Thr Ile
115 120 125

cat atc caa tac ctt gat agt gaa ctt gca tat ctc cct ctt tcc tta 432
His Ile Gln Tyr Leu Asp Ser Glu Leu Ala Tyr Leu Pro Leu Ser Leu
130 135 140

gaa gaa ttc aat ctc tct aaa ggt aat cct gct ctt gct gtt aat atc 480
Glu Glu Phe Asn Leu Ser Lys Gly Asn Pro Ala Leu Ala Val Asn Ile
145 150 155 160

cta tcc tct gta agt agc ctc atg caa tat ata cat aat aat gga tat 528
Leu Ser Ser Val Ser Ser Leu Met Gln Tyr Ile His Asn Asn Gly Tyr
165 170 175

cca tta gcc aaa ata aaa aaa act caa tac ata att aat cgg atg gat 576
Pro Leu Ala Lys Ile Lys Lys Thr Gln Tyr Ile Ile Asn Arg Met Asp
180 185 190

tat aca ttt gat att gat tta gta ata aga caa gga ccg tta ctc cat 624
Tyr Thr Phe Asp Ile Asp Leu Val Ile Arg Gln Gly Pro Leu Leu His
195 200 205

atg ggt aaa gta caa cct caa cat aat ctc aat att tca aca ata ttc 672
Met Gly Lys Val Gln Pro Gln His Asn Leu Asn Ile Ser Thr Ile Phe
210 215 220

cta aat aaa att gct aca tgg aag gaa gga agg gta tgg aac aat gca 720
Leu Asn Lys Ile Ala Thr Trp Lys Glu Gly Arg Val Trp Asn Asn Ala
225 230 235 240

ctc ctt gat tct tat cga aca cgg ctt caa caa aca ggc ctt ttc agt 768
Leu Leu Asp Ser Tyr Arg Thr Arg Leu Gln Gln Thr Gly Leu Phe Ser
245 250 255

```
tct ata act ctc aat cca agg aat caa aaa gaa caa aat ggt aac acc   816
Ser Ile Thr Leu Asn Pro Arg Asn Gln Lys Glu Gln Asn Gly Asn Thr
            260                 265                 270

tct ata gaa ctt gtt gca aca gaa gcc cct cca agg act att agt ggt   864
Ser Ile Glu Leu Val Ala Thr Glu Ala Pro Pro Arg Thr Ile Ser Gly
        275                 280                 285

ggc tta caa tac tct tct gat caa ggt att ggt gca cgt ggg act tgg   912
Gly Leu Gln Tyr Ser Ser Asp Gln Gly Ile Gly Ala Arg Gly Thr Trp
    290                 295                 300

gaa cat cga aat gtt ttt ggt aat gga gaa ctt ttt cgt ata aca gca   960
Glu His Arg Asn Val Phe Gly Asn Gly Glu Leu Phe Arg Ile Thr Ala
305                 310                 315                 320

cca ata agt cga gat gat caa aaa att atg gca aac ttc caa aaa cca   1008
Pro Ile Ser Arg Asp Asp Gln Lys Ile Met Ala Asn Phe Gln Lys Pro
                325                 330                 335

gcc ttt ggc cgt cca aat caa tca tta att agt gaa gca caa ctt aaa   1056
Ala Phe Gly Arg Pro Asn Gln Ser Leu Ile Ser Glu Ala Gln Leu Lys
            340                 345                 350

aaa gaa aat aca aaa agt tac aaa caa caa ctt gca tct att gct tta   1104
Lys Glu Asn Thr Lys Ser Tyr Lys Gln Gln Leu Ala Ser Ile Ala Leu
        355                 360                 365

gga att gaa cga caa ttt aat aga cgt tgg ttt ggt agt agc agt ctt   1152
Gly Ile Glu Arg Gln Phe Asn Arg Arg Trp Phe Gly Ser Ser Ser Leu
    370                 375                 380

tca gtt gat aca gga ttt atg gat gat cga gat tct ata aaa aaa ata   1200
Ser Val Asp Thr Gly Phe Met Asp Asp Arg Asp Ser Ile Lys Lys Ile
385                 390                 395                 400

ttt act ctt ttt ggc atc ccc tta tca ata aca agg gat agt tct aaa   1248
Phe Thr Leu Phe Gly Ile Pro Leu Ser Ile Thr Arg Asp Ser Ser Lys
                405                 410                 415

gat cct ctt aat cct atc caa gga aca aaa gct acc tta aat gtt act   1296
Asp Pro Leu Asn Pro Ile Gln Gly Thr Lys Ala Thr Leu Asn Val Thr
            420                 425                 430
```

```
cct tat att ggt aaa tat aaa aaa aag att ttg act tta cgt agt cgg   1344
Pro Tyr Ile Gly Lys Tyr Lys Lys Lys Ile Leu Thr Leu Arg Ser Arg
        435                 440                 445

ttt gat ttt agc ttt tac ata gac gtt ctt aaa aca ggg aaa ctt atc   1392
Phe Asp Phe Ser Phe Tyr Ile Asp Val Leu Lys Thr Gly Lys Leu Ile
    450                 455                 460

ttg gct aac aaa ata gca ata ggt tcc ctc cta ggg aaa gat ata gaa   1440
Leu Ala Asn Lys Ile Ala Ile Gly Ser Leu Leu Gly Lys Asp Ile Glu
465                 470                 475                 480

aac tat cct gca ata cta agg ttt tat gct ggg ggt ggt ggt agt gta   1488
Asn Tyr Pro Ala Ile Leu Arg Phe Tyr Ala Gly Gly Gly Gly Ser Val
                485                 490                 495

aga ggg tat gac tat caa tca ttg gga cca aaa aat aaa tat ggg gat   1536
Arg Gly Tyr Asp Tyr Gln Ser Leu Gly Pro Lys Asn Lys Tyr Gly Asp
            500                 505                 510

gct att gga gga ctt tct ttt tca act att agt ttt gaa tta cga tta   1584
Ala Ile Gly Gly Leu Ser Phe Ser Thr Ile Ser Phe Glu Leu Arg Leu
        515                 520                 525

aaa ata aca gaa tcc att ggc att gtg cca att tat tgg atg ggg gaa   1632
Lys Ile Thr Glu Ser Ile Gly Ile Val Pro Ile Tyr Trp Met Gly Glu
    530                 535                 540

tat tta cga aaa aaa aat ttc ctg act tta aaa aaa tca ata tat tgg   1680
Tyr Leu Arg Lys Lys Asn Phe Leu Thr Leu Lys Lys Ser Ile Tyr Trp
545                 550                 555                 560

ggg gta ggc ctg ggg cta cga tat tat aca agt ttt gcc ccc ata cgt   1728
Gly Val Gly Leu Gly Leu Arg Tyr Tyr Thr Ser Phe Ala Pro Ile Arg
                565                 570                 575

tta gat ata gca act cca ctt caa gat aga agc cat aat aaa cac ttt   1776
Leu Asp Ile Ala Thr Pro Leu Gln Asp Arg Ser His Asn Lys His Phe
            580                 585                 590

caa ctt tat att agt att ggg caa gca ttc taa tga                  1812
Gln Leu Tyr Ile Ser Ile Gly Gln Ala Phe
        595                 600
```

<210> 16
<211> 602
<212> PRT
<213> Lawsonia intracellularis

<400> 16

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Met Gln Lys Val Cys Tyr Phe Phe Leu Ile Thr Phe Phe Tyr Phe Phe
 1               5                  10                  15

Ile Thr Glu Asn Tyr Leu Phe Ala Thr Ser Ile Thr Thr Ser Thr Ile
            20                  25                  30

Asn Gln Gln His Ile Ala Tyr Thr Val Thr Phe Thr Ser Pro Glu Asn
        35                  40                  45

Pro Asn Leu Ala Thr Glu Met Glu Thr His Ser Glu Leu Val Lys Leu
    50                  55                  60

Ala Asn Gln Ser Leu Asp Ser Lys Ile Gly Leu Asn Leu Arg Val Lys
65                  70                  75                  80

Glu Asp Ile Ser Thr Ala Gln Lys Ile Leu Asp Ser Asn Gly Tyr Tyr
                85                  90                  95

Ser Gly Ser Val Glu Gly Lys Ile Asp Trp Gln Thr Asn Pro Ile Ser
            100                 105                 110

Ile Gln Ile Gln Phe Lys Pro Asn Val Gln Tyr Lys Ile Asn Thr Ile
        115                 120                 125

His Ile Gln Tyr Leu Asp Ser Glu Leu Ala Tyr Leu Pro Leu Ser Leu
    130                 135                 140

Glu Glu Phe Asn Leu Ser Lys Gly Asn Pro Ala Leu Ala Val Asn Ile
145                 150                 155                 160

Leu Ser Ser Val Ser Ser Leu Met Gln Tyr Ile His Asn Asn Gly Tyr
                165                 170                 175

Pro Leu Ala Lys Ile Lys Lys Thr Gln Tyr Ile Ile Asn Arg Met Asp
            180                 185                 190

Tyr Thr Phe Asp Ile Asp Leu Val Ile Arg Gln Gly Pro Leu Leu His
        195                 200                 205
```

Met Gly Lys Val Gln Pro Gln His Asn Leu Asn Ile Ser Thr Ile Phe
210 215 220

Leu Asn Lys Ile Ala Thr Trp Lys Glu Gly Arg Val Trp Asn Asn Ala
225 230 235 240

Leu Leu Asp Ser Tyr Arg Thr Arg Leu Gln Gln Thr Gly Leu Phe Ser
245 250 255

Ser Ile Thr Leu Asn Pro Arg Asn Gln Lys Glu Gln Asn Gly Asn Thr
260 265 270

Ser Ile Glu Leu Val Ala Thr Glu Ala Pro Pro Arg Thr Ile Ser Gly
275 280 285

Gly Leu Gln Tyr Ser Ser Asp Gln Gly Ile Gly Ala Arg Gly Thr Trp
290 295 300

Glu His Arg Asn Val Phe Gly Asn Gly Glu Leu Phe Arg Ile Thr Ala
305 310 315 320

Pro Ile Ser Arg Asp Asp Gln Lys Ile Met Ala Asn Phe Gln Lys Pro
325 330 335

Ala Phe Gly Arg Pro Asn Gln Ser Leu Ile Ser Glu Ala Gln Leu Lys
340 345 350

Lys Glu Asn Thr Lys Ser Tyr Lys Gln Gln Leu Ala Ser Ile Ala Leu
355 360 365

Gly Ile Glu Arg Gln Phe Asn Arg Arg Trp Phe Gly Ser Ser Ser Leu
370 375 380

Ser Val Asp Thr Gly Phe Met Asp Asp Arg Asp Ser Ile Lys Lys Ile
385 390 395 400

Phe Thr Leu Phe Gly Ile Pro Leu Ser Ile Thr Arg Asp Ser Ser Lys
405 410 415

Asp Pro Leu Asn Pro Ile Gln Gly Thr Lys Ala Thr Leu Asn Val Thr
420 425 430

Pro Tyr Ile Gly Lys Tyr Lys Lys Lys Ile Leu Thr Leu Arg Ser Arg
435 440 445

```
Phe Asp Phe Ser Phe Tyr Ile Asp Val Leu Lys Thr Gly Lys Leu Ile
    450                 455                 460

Leu Ala Asn Lys Ile Ala Ile Gly Ser Leu Leu Gly Lys Asp Ile Glu
465                 470                 475                 480

Asn Tyr Pro Ala Ile Leu Arg Phe Tyr Ala Gly Gly Gly Gly Ser Val
                485                 490                 495

Arg Gly Tyr Asp Tyr Gln Ser Leu Gly Pro Lys Asn Lys Tyr Gly Asp
            500                 505                 510

Ala Ile Gly Gly Leu Ser Phe Ser Thr Ile Ser Phe Glu Leu Arg Leu
        515                 520                 525

Lys Ile Thr Glu Ser Ile Gly Ile Val Pro Ile Tyr Trp Met Gly Glu
    530                 535                 540

Tyr Leu Arg Lys Lys Asn Phe Leu Thr Leu Lys Lys Ser Ile Tyr Trp
545                 550                 555                 560

Gly Val Gly Leu Gly Leu Arg Tyr Tyr Thr Ser Phe Ala Pro Ile Arg
                565                 570                 575

Leu Asp Ile Ala Thr Pro Leu Gln Asp Arg Ser His Asn Lys His Phe
            580                 585                 590

Gln Leu Tyr Ile Ser Ile Gly Gln Ala Phe
        595                 600
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<210> 17
<211> 4149
<212> DNA
<213> Lawsonia intracellularis

<220>
<221> CDS
<222> (1)..(4146)

<400> 17

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atg aat aac aca aaa ata ctt tct aag tta ctc tat acc ctc tta gga   48
Met Asn Asn Thr Lys Ile Leu Ser Lys Leu Leu Tyr Thr Leu Leu Gly
  1               5                  10                  15

gca ttt acg tta ttt tta gga ctt att att aca ggc att ctt ttt ata   96
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Ala Phe Thr Leu Phe Leu Gly Leu Ile Ile Thr Gly Ile Leu Phe Ile
                20                  25                  30

cgg acc tct aca ggc att gct tgg att aaa aat aca gtt tct tct tta   144
Arg Thr Ser Thr Gly Ile Ala Trp Ile Lys Asn Thr Val Ser Ser Leu
          35                  40                  45

ctt caa caa caa gga att ata cta caa gta tct tca att att gga cca   192
Leu Gln Gln Gln Gly Ile Ile Leu Gln Val Ser Ser Ile Ile Gly Pro
     50                  55                  60

ttc cca gaa caa att act att aat gaa ctt agc ctt agt gat gtg aat   240
Phe Pro Glu Gln Ile Thr Ile Asn Glu Leu Ser Leu Ser Asp Val Asn
 65                  70                  75                  80

gga act tac ctt aca ata tct aac tta gaa atc caa tca aac tta tgg   288
Gly Thr Tyr Leu Thr Ile Ser Asn Leu Glu Ile Gln Ser Asn Leu Trp
                85                  90                  95

gct tta ttc aaa ggt caa ctt gaa att ctg tct ttt gaa ctt aat gat   336
Ala Leu Phe Lys Gly Gln Leu Glu Ile Leu Ser Phe Glu Leu Asn Asp
            100                 105                 110

ctt gta tta tat cgc tta ccc tca aat aat aat cta aaa aaa tca tct   384
Leu Val Leu Tyr Arg Leu Pro Ser Asn Asn Asn Leu Lys Lys Ser Ser
        115                 120                 125

aca agt ttt gtg tta cct cac ata tca ttt gat tta act cca tgg tgg   432
Thr Ser Phe Val Leu Pro His Ile Ser Phe Asp Leu Thr Pro Trp Trp
    130                 135                 140

act gaa cat att cgt att caa aac atc cat att aac aat aca caa ctt   480
Thr Glu His Ile Arg Ile Gln Asn Ile His Ile Asn Asn Thr Gln Leu
145                 150                 155                 160

tcc tct gat att ata ggt att cca ttg gta tta tcc ctt gag ggt gat   528
Ser Ser Asp Ile Ile Gly Ile Pro Leu Val Leu Ser Leu Glu Gly Asp
                165                 170                 175

ggt aca tta aca aat tgg aat gga aca ttt caa cta tcc tct tct aac   576
Gly Thr Leu Thr Asn Trp Asn Gly Thr Phe Gln Leu Ser Ser Ser Asn
            180                 185                 190

aaa aca aaa att ata gga acg ctt cgt tac caa ggg aat aag aca caa   624
Lys Thr Lys Ile Ile Gly Thr Leu Arg Tyr Gln Gly Asn Lys Thr Gln
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          195                 200                 205

ttt ttt gaa tat gtt cat cct aca cgg ata gta aca cta gag ata gac   672
Phe Phe Glu Tyr Val His Pro Thr Arg Ile Val Thr Leu Glu Ile Asp
    210                 215                 220

agc gta gct gat aaa aag tca tat aat aat agt atc ctt gaa caa cct   720
Ser Val Ala Asp Lys Lys Ser Tyr Asn Asn Ser Ile Leu Glu Gln Pro
225                 230                 235                 240

cta cat tta cac ctt tct att tat cct gaa cat aat aga att atc tta   768
Leu His Leu His Leu Ser Ile Tyr Pro Glu His Asn Arg Ile Ile Leu
                245                 250                 255

cac tca tta cta gct gaa tat ggt agc tgg tta ctt aca tca gaa agt   816
His Ser Leu Leu Ala Glu Tyr Gly Ser Trp Leu Leu Thr Ser Glu Ser
            260                 265                 270

att gaa gta tct aat gag caa tta aaa gga aat att tta tta aaa tat   864
Ile Glu Val Ser Asn Glu Gln Leu Lys Gly Asn Ile Leu Leu Lys Tyr
        275                 280                 285

aat gga gaa gct act cat caa ctt cct ata aaa aaa ctt aac tca tca   912
Asn Gly Glu Ala Thr His Gln Leu Pro Ile Lys Lys Leu Asn Ser Ser
    290                 295                 300

att acc ctc agt ggc tca cta aat aaa cct aat ttt agt ata caa atg   960
Ile Thr Leu Ser Gly Ser Leu Asn Lys Pro Asn Phe Ser Ile Gln Met
305                 310                 315                 320

aca tta cct gaa att aac att aca aaa aac ata ata gat ctt caa aca  1008
Thr Leu Pro Glu Ile Asn Ile Thr Lys Asn Ile Ile Asp Leu Gln Thr
                325                 330                 335

gaa ctt gtt att aat cta gga ctt ttc tct act cac tct gat att ctt  1056
Glu Leu Val Ile Asn Leu Gly Leu Phe Ser Thr His Ser Asp Ile Leu
            340                 345                 350

aca tct ggg aca att aca gta cag gga gaa act ata ccc aat agt att  1104
Thr Ser Gly Thr Ile Thr Val Gln Gly Glu Thr Ile Pro Asn Ser Ile
        355                 360                 365

ctt tcc agt gca gtt gat ata ata gcc tct aca aca aca cat aca att  1152
Leu Ser Ser Ala Val Asp Ile Ile Ala Ser Thr Thr Thr His Thr Ile
    370                 375                 380
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acc tta gag cat gca acc tta aca tct cca gaa atg cat ttt tcc cta   1200
Thr Leu Glu His Ala Thr Leu Thr Ser Pro Glu Met His Phe Ser Leu
385                 390                 395                 400

tct gga gaa ttt aat agt ctt cta gga aat atc gat gca aac cta aaa   1248
Ser Gly Glu Phe Asn Ser Leu Leu Gly Asn Ile Asp Ala Asn Leu Lys
                405                 410                 415

ggt aat act cca act ctt agt ata ttt tct tct ctt ctt gga cta cct   1296
Gly Asn Thr Pro Thr Leu Ser Ile Phe Ser Ser Leu Leu Gly Leu Pro
            420                 425                 430

gat ctt act ggg caa agt aac att act ata gga tta cac cgt caa ggg   1344
Asp Leu Thr Gly Gln Ser Asn Ile Thr Ile Gly Leu His Arg Gln Gly
        435                 440                 445

tct tcc tct tca ata gaa gga aca gca act gtc tca ctt aat aat atg   1392
Ser Ser Ser Ser Ile Glu Gly Thr Ala Thr Val Ser Leu Asn Asn Met
    450                 455                 460

aac tgg gga gta caa gca tta cag ggg aca tta ggt gat aat gca act   1440
Asn Trp Gly Val Gln Ala Leu Gln Gly Thr Leu Gly Asp Asn Ala Thr
465                 470                 475                 480

cta agt gga ata tat aat tta act ccc ata gac tgg tct att tct tta   1488
Leu Ser Gly Ile Tyr Asn Leu Thr Pro Ile Asp Trp Ser Ile Ser Leu
                485                 490                 495

aac aaa ttg aaa tta aca gca aag aat gtt tat gct gaa ggc ctt att   1536
Asn Lys Leu Lys Leu Thr Ala Lys Asn Val Tyr Ala Glu Gly Leu Ile
            500                 505                 510

aat ttt caa aaa aaa tac ata gat agc tct ata aat ctt ata att cct   1584
Asn Phe Gln Lys Lys Tyr Ile Asp Ser Ser Ile Asn Leu Ile Ile Pro
        515                 520                 525

aac ctt cag cta ata gct cct cct ata tct gga gag tta caa tcc tta   1632
Asn Leu Gln Leu Ile Ala Pro Pro Ile Ser Gly Glu Leu Gln Ser Leu
    530                 535                 540

att aca gtg tct gga aaa ctt gac gca cct tct ata gaa agc aaa att   1680
Ile Thr Val Ser Gly Lys Leu Asp Ala Pro Ser Ile Glu Ser Lys Ile
545                 550                 555                 560
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ttt tca tca caa ctc acc tgg aat gcg ctc caa ctt aat aat cct caa   1728
Phe Ser Ser Gln Leu Thr Trp Asn Ala Leu Gln Leu Asn Asn Pro Gln
                565                 570                 575

ctc ata ata act act act caa tct tct tcc tct gcg att aaa ggt aat   1776
Leu Ile Ile Thr Thr Thr Gln Ser Ser Ser Ser Ala Ile Lys Gly Asn
            580                 585                 590

ata aca ctc tcg gct gag cca gct tca tct gag gca tta acc ttt tca   1824
Ile Thr Leu Ser Ala Glu Pro Ala Ser Ser Glu Ala Leu Thr Phe Ser
        595                 600                 605

agt aat tgg gga atc cta cct acg gaa ata cta gta gaa aaa att ata   1872
Ser Asn Trp Gly Ile Leu Pro Thr Glu Ile Leu Val Glu Lys Ile Ile
    610                 615                 620

gga aat ata tta gga gta aat ctt gat ggt aat att aaa ata aca aaa   1920
Gly Asn Ile Leu Gly Val Asn Leu Asp Gly Asn Ile Lys Ile Thr Lys
625                 630                 635                 640

aaa gat tac ctt ata aat ggt gat att att gca gaa gtt cag tct tgg   1968
Lys Asp Tyr Leu Ile Asn Gly Asp Ile Ile Ala Glu Val Gln Ser Trp
                645                 650                 655

aaa gat att gca aac ata ttg caa ata cct att aga ggt tca gca tca   2016
Lys Asp Ile Ala Asn Ile Leu Gln Ile Pro Ile Arg Gly Ser Ala Ser
            660                 665                 670

ata aaa ata cag ttt gat cca aag aat caa caa tgt att tct act caa   2064
Ile Lys Ile Gln Phe Asp Pro Lys Asn Gln Gln Cys Ile Ser Thr Gln
        675                 680                 685

tgg caa tta aaa aat ttc ata tta ggt aat aat ttt aat gta act act   2112
Trp Gln Leu Lys Asn Phe Ile Leu Gly Asn Asn Phe Asn Val Thr Thr
    690                 695                 700

ata aaa gga aga gca gat aca ata caa ctt cat aag aat cct aca att   2160
Ile Lys Gly Arg Ala Asp Thr Ile Gln Leu His Lys Asn Pro Thr Ile
705                 710                 715                 720

gct ctc tct tca aaa att ggt gct ggt aca tat gaa gac ttt caa tgg   2208
Ala Leu Ser Ser Lys Ile Gly Ala Gly Thr Tyr Glu Asp Phe Gln Trp
                725                 730                 735

aca caa ggg acg tta gac ata aaa ggc aca tta aaa aat ttt aat agt   2256
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```
Thr Gln Gly Thr Leu Asp Ile Lys Gly Thr Leu Lys Asn Phe Asn Ser
            740                 745                 750

aaa ata aat ata gca gga caa aca act gta aac gca aac ttt caa aca   2304
Lys Ile Asn Ile Ala Gly Gln Thr Thr Val Asn Ala Asn Phe Gln Thr
        755                 760                 765

aat ctt ttt gaa aaa aat att aat ata act act ctt aat tta aaa aat   2352
Asn Leu Phe Glu Lys Asn Ile Asn Ile Thr Thr Leu Asn Leu Lys Asn
    770                 775                 780

att caa aaa aat ata gga att aag ctc ctt cag cca ata aaa att ata   2400
Ile Gln Lys Asn Ile Gly Ile Lys Leu Leu Gln Pro Ile Lys Ile Ile
785                 790                 795                 800

gtc tca cct caa caa ttt gtt ctt aat aac tgt tca cta gca att ctt   2448
Val Ser Pro Gln Gln Phe Val Leu Asn Asn Cys Ser Leu Ala Ile Leu
                805                 810                 815

cca tct gga aca att aca act gat ata tat gtt act cct caa cga ctt   2496
Pro Ser Gly Thr Ile Thr Thr Asp Ile Tyr Val Thr Pro Gln Arg Leu
            820                 825                 830

aat gct aat gca atc att aaa gaa gtt tca ctt ctc tct ttc caa cca   2544
Asn Ala Asn Ala Ile Ile Lys Glu Val Ser Leu Leu Ser Phe Gln Pro
        835                 840                 845

ttt agt ata ctt ctt cct caa gga aat ata aat gga cac ata aca ctt   2592
Phe Ser Ile Leu Leu Pro Gln Gly Asn Ile Asn Gly His Ile Thr Leu
    850                 855                 860

aca gga ata cct agt aaa cct aaa gga aca ctc tca ttt gat att cta   2640
Thr Gly Ile Pro Ser Lys Pro Lys Gly Thr Leu Ser Phe Asp Ile Leu
865                 870                 875                 880

aac ata cat tat cca agg cca aat cca tca ata gca aac tta cat gta   2688
Asn Ile His Tyr Pro Arg Pro Asn Pro Ser Ile Ala Asn Leu His Val
                885                 890                 895

gaa ggg gaa att ata tct tct cct aac aat ata tgt aaa ctt aat gca   2736
Glu Gly Glu Ile Ile Ser Ser Pro Asn Asn Ile Cys Lys Leu Asn Ala
            900                 905                 910

acc cta aca gaa aaa aaa gag cct ata cct ata tca ata caa gca aca   2784
Thr Leu Thr Glu Lys Lys Glu Pro Ile Pro Ile Ser Ile Gln Ala Thr
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          915                 920                 925

    ctc cct ttt gag ttc aca gaa aac aat atc cct atg cta tct aaa atg   2832
    Leu Pro Phe Glu Phe Thr Glu Asn Asn Ile Pro Met Leu Ser Lys Met
5        930                 935                 940

    agg cct ttt tct gcc cat atc aag tgg act gga ata tta gat aca ctt   2880
    Arg Pro Phe Ser Ala His Ile Lys Trp Thr Gly Ile Leu Asp Thr Leu
    945                 950                 955                 960
10
    tgg aaa ctc att cca ctt act gat tac att atg gct ggg aat gga tct   2928
    Trp Lys Leu Ile Pro Leu Thr Asp Tyr Ile Met Ala Gly Asn Gly Ser
                    965                 970                 975

15  tta gat gct tct ctt tct ggg act tta gat agt cca aca tat gca att   2976
    Leu Asp Ala Ser Leu Ser Gly Thr Leu Asp Ser Pro Thr Tyr Ala Ile
                980                 985                 990

    ata aca aca ctt tct aat gct aac ttt caa gat ctc tcc ctt ggt ctt   3024
20  Ile Thr Thr Leu Ser Asn Ala Asn Phe Gln Asp Leu Ser Leu Gly Leu
          995                1000                1005

    tac tta gaa aat atc aat gct aaa tta cag gtc ttt tct aat aga atc   3072
    Tyr Leu Glu Asn Ile Asn Ala Lys Leu Gln Val Phe Ser Asn Arg Ile
25     1010                1015                1020

    tcc cat att caa gct aca gca tct gat ggt aaa caa ggt agt ata caa   3120
    Ser His Ile Gln Ala Thr Ala Ser Asp Gly Lys Gln Gly Ser Ile Gln
    1025                1030                1035                1040
30
    ctt att ggt aat att ggc tca tct aaa gaa cac ttt cct ttg tct att   3168
    Leu Ile Gly Asn Ile Gly Ser Ser Lys Glu His Phe Pro Leu Ser Ile
                    1045                1050                1055

35  aat ggc tcc ttt aca aac ctt gct cca tta caa cgt aaa gac cta agt   3216
    Asn Gly Ser Phe Thr Asn Leu Ala Pro Leu Gln Arg Lys Asp Leu Ser
               1060                1065                1070

    ctt aca ctt tca gga gca gct act ctt gaa gga aca tta aaa cag tct   3264
40  Leu Thr Leu Ser Gly Ala Ala Thr Leu Glu Gly Thr Leu Lys Gln Ser
          1075                1080                1085

    gaa gtt aaa ggc gat att gtt att aac caa ggc gaa ttt caa ctt act   3312
    Glu Val Lys Gly Asp Ile Val Ile Asn Gln Gly Glu Phe Gln Leu Thr
45     1090                1095                1100
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gaa ggg tta acc agt aat att cca act ctt aat gta gtt gat agc act 3360
Glu Gly Leu Thr Ser Asn Ile Pro Thr Leu Asn Val Val Asp Ser Thr
1105 1110 1115 1120

caa caa caa aat aca aag acc aaa aaa gct acc tat caa caa cct acc 3408
Gln Gln Gln Asn Thr Lys Thr Lys Lys Ala Thr Tyr Gln Gln Pro Thr
1125 1130 1135

tta tct att gcg tta agt atc ccg aat cgt ttt ttt gtc cgt agt agt 3456
Leu Ser Ile Ala Leu Ser Ile Pro Asn Arg Phe Phe Val Arg Ser Ser
1140 1145 1150

atg ttt gaa agt gag tgg gga ggg aac cta act att aac aaa gtc ata 3504
Met Phe Glu Ser Glu Trp Gly Gly Asn Leu Thr Ile Asn Lys Val Ile
1155 1160 1165

aca agt cct gtt att aca gga gca cta act tct ata aga gga aat ttt 3552
Thr Ser Pro Val Ile Thr Gly Ala Leu Thr Ser Ile Arg Gly Asn Phe
1170 1175 1180

aat tta cta gga aaa caa ttt tct ctt gct aaa agt aca ata tca ttt 3600
Asn Leu Leu Gly Lys Gln Phe Ser Leu Ala Lys Ser Thr Ile Ser Phe
1185 1190 1195 1200

tca gga tca gtt cca cca aac cca cta ctc aat att tct tta aca tat 3648
Ser Gly Ser Val Pro Pro Asn Pro Leu Leu Asn Ile Ser Leu Thr Tyr
1205 1210 1215

tca tca cct tct att aca gct ata ggc att att aaa ggt aca act agt 3696
Ser Ser Pro Ser Ile Thr Ala Ile Gly Ile Ile Lys Gly Thr Thr Ser
1220 1225 1230

aat cct aat att act ttt tca agt aca cca cct tta cct caa gat gaa 3744
Asn Pro Asn Ile Thr Phe Ser Ser Thr Pro Pro Leu Pro Gln Asp Glu
1235 1240 1245

ata gtt tcc caa gtt ctt ttt ggt aaa agc tca caa agt ctt agc agg 3792
Ile Val Ser Gln Val Leu Phe Gly Lys Ser Ser Gln Ser Leu Ser Arg
1250 1255 1260

ata caa gcc ata caa ctt gct caa gaa tta gca aac tta aca gga ttt 3840
Ile Gln Ala Ile Gln Leu Ala Gln Glu Leu Ala Asn Leu Thr Gly Phe
1265 1270 1275 1280

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aat act gga agt atg aat ttc cta aca aat att cga cag aca tta ggg   3888
Asn Thr Gly Ser Met Asn Phe Leu Thr Asn Ile Arg Gln Thr Leu Gly
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tta gat ata ctt agc tta ggg aca act tct aat aga aaa gcc aat aca   3936
Leu Asp Ile Leu Ser Leu Gly Thr Thr Ser Asn Arg Lys Ala Asn Thr
          1300                1305                1310

tcc aac tca aac gat caa ata gaa gat atc cct gtt ata gaa cta ggt   3984
Ser Asn Ser Asn Asp Gln Ile Glu Asp Ile Pro Val Ile Glu Leu Gly
      1315                1320                1325

aaa tat att aca gac act gtt tat gtt ggt gtt gaa caa agt tat tta   4032
Lys Tyr Ile Thr Asp Thr Val Tyr Val Gly Val Glu Gln Ser Tyr Leu
   1330                1335                1340

gat agt aat gat act ggg gca aga ata tca gtt gaa ctt gca cct aat   4080
Asp Ser Asn Asp Thr Gly Ala Arg Ile Ser Val Glu Leu Ala Pro Asn
1345                1350                1355                1360

ttt aat ctt gaa ggt aga aca ggg act caa tat agt gag ata ggt att   4128
Phe Asn Leu Glu Gly Arg Thr Gly Thr Gln Tyr Ser Glu Ile Gly Ile
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aat tgg aaa aaa gat tat taa                                      4149
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<400> 18

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Ala Phe Thr Leu Phe Leu Gly Leu Ile Ile Thr Gly Ile Leu Phe Ile
             20                  25                  30

Arg Thr Ser Thr Gly Ile Ala Trp Ile Lys Asn Thr Val Ser Ser Leu
         35                  40                  45

Leu Gln Gln Gln Gly Ile Ile Leu Gln Val Ser Ser Ile Ile Gly Pro
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50 55 60

Phe Pro Glu Gln Ile Thr Ile Asn Glu Leu Ser Leu Ser Asp Val Asn
65 70 75 80

Gly Thr Tyr Leu Thr Ile Ser Asn Leu Glu Ile Gln Ser Asn Leu Trp
85 90 95

Ala Leu Phe Lys Gly Gln Leu Glu Ile Leu Ser Phe Glu Leu Asn Asp
100 105 110

Leu Val Leu Tyr Arg Leu Pro Ser Asn Asn Asn Leu Lys Lys Ser Ser
115 120 125

Thr Ser Phe Val Leu Pro His Ile Ser Phe Asp Leu Thr Pro Trp Trp
130 135 140

Thr Glu His Ile Arg Ile Gln Asn Ile His Ile Asn Asn Thr Gln Leu
145 150 155 160

Ser Ser Asp Ile Ile Gly Ile Pro Leu Val Leu Ser Leu Glu Gly Asp
165 170 175

Gly Thr Leu Thr Asn Trp Asn Gly Thr Phe Gln Leu Ser Ser Ser Asn
180 185 190

Lys Thr Lys Ile Ile Gly Thr Leu Arg Tyr Gln Gly Asn Lys Thr Gln
195 200 205

Phe Phe Glu Tyr Val His Pro Thr Arg Ile Val Thr Leu Glu Ile Asp
210 215 220

Ser Val Ala Asp Lys Lys Ser Tyr Asn Asn Ser Ile Leu Glu Gln Pro
225 230 235 240

Leu His Leu His Leu Ser Ile Tyr Pro Glu His Asn Arg Ile Ile Leu
245 250 255

His Ser Leu Leu Ala Glu Tyr Gly Ser Trp Leu Leu Thr Ser Glu Ser
260 265 270

Ile Glu Val Ser Asn Glu Gln Leu Lys Gly Asn Ile Leu Leu Lys Tyr
275 280 285

Asn Gly Glu Ala Thr His Gln Leu Pro Ile Lys Lys Leu Asn Ser Ser

290 295 300

Ile Thr Leu Ser Gly Ser Leu Asn Lys Pro Asn Phe Ser Ile Gln Met
305 310 315 320

Thr Leu Pro Glu Ile Asn Ile Thr Lys Asn Ile Ile Asp Leu Gln Thr
325 330 335

Glu Leu Val Ile Asn Leu Gly Leu Phe Ser Thr His Ser Asp Ile Leu
340 345 350

Thr Ser Gly Thr Ile Thr Val Gln Gly Glu Thr Ile Pro Asn Ser Ile
355 360 365

Leu Ser Ser Ala Val Asp Ile Ile Ala Ser Thr Thr Thr His Thr Ile
370 375 380

Thr Leu Glu His Ala Thr Leu Thr Ser Pro Glu Met His Phe Ser Leu
385 390 395 400

Ser Gly Glu Phe Asn Ser Leu Leu Gly Asn Ile Asp Ala Asn Leu Lys
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Gly Asn Thr Pro Thr Leu Ser Ile Phe Ser Ser Leu Leu Gly Leu Pro
420 425 430

Asp Leu Thr Gly Gln Ser Asn Ile Thr Ile Gly Leu His Arg Gln Gly
435 440 445

Ser Ser Ser Ser Ile Glu Gly Thr Ala Thr Val Ser Leu Asn Asn Met
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Leu Ser Gly Ile Tyr Asn Leu Thr Pro Ile Asp Trp Ser Ile Ser Leu
485 490 495

Asn Lys Leu Lys Leu Thr Ala Lys Asn Val Tyr Ala Glu Gly Leu Ile
500 505 510

Asn Phe Gln Lys Lys Tyr Ile Asp Ser Ser Ile Asn Leu Ile Ile Pro
515 520 525

Asn Leu Gln Leu Ile Ala Pro Pro Ile Ser Gly Glu Leu Gln Ser Leu

530 535 540

Ile Thr Val Ser Gly Lys Leu Asp Ala Pro Ser Ile Glu Ser Lys Ile
545 550 555 560

Phe Ser Ser Gln Leu Thr Trp Asn Ala Leu Gln Leu Asn Asn Pro Gln
565 570 575

Leu Ile Ile Thr Thr Thr Gln Ser Ser Ser Ser Ala Ile Lys Gly Asn
580 585 590

Ile Thr Leu Ser Ala Glu Pro Ala Ser Ser Glu Ala Leu Thr Phe Ser
595 600 605

Ser Asn Trp Gly Ile Leu Pro Thr Glu Ile Leu Val Glu Lys Ile Ile
610 615 620

Gly Asn Ile Leu Gly Val Asn Leu Asp Gly Asn Ile Lys Ile Thr Lys
625 630 635 640

Lys Asp Tyr Leu Ile Asn Gly Asp Ile Ile Ala Glu Val Gln Ser Trp
645 650 655

Lys Asp Ile Ala Asn Ile Leu Gln Ile Pro Ile Arg Gly Ser Ala Ser
660 665 670

Ile Lys Ile Gln Phe Asp Pro Lys Asn Gln Gln Cys Ile Ser Thr Gln
675 680 685

Trp Gln Leu Lys Asn Phe Ile Leu Gly Asn Asn Phe Asn Val Thr Thr
690 695 700

Ile Lys Gly Arg Ala Asp Thr Ile Gln Leu His Lys Asn Pro Thr Ile
705 710 715 720

Ala Leu Ser Ser Lys Ile Gly Ala Gly Thr Tyr Glu Asp Phe Gln Trp
725 730 735

Thr Gln Gly Thr Leu Asp Ile Lys Gly Thr Leu Lys Asn Phe Asn Ser
740 745 750

Lys Ile Asn Ile Ala Gly Gln Thr Thr Val Asn Ala Asn Phe Gln Thr
755 760 765

Asn Leu Phe Glu Lys Asn Ile Asn Ile Thr Thr Leu Asn Leu Lys Asn

770 775 780

Ile Gln Lys Asn Ile Gly Ile Lys Leu Leu Gln Pro Ile Lys Ile Ile
785 790 795 800

Val Ser Pro Gln Gln Phe Val Leu Asn Asn Cys Ser Leu Ala Ile Leu
805 810 815

Pro Ser Gly Thr Ile Thr Thr Asp Ile Tyr Val Thr Pro Gln Arg Leu
820 825 830

Asn Ala Asn Ala Ile Ile Lys Glu Val Ser Leu Leu Ser Phe Gln Pro
835 840 845

Phe Ser Ile Leu Leu Pro Gln Gly Asn Ile Asn Gly His Ile Thr Leu
850 855 860

Thr Gly Ile Pro Ser Lys Pro Lys Gly Thr Leu Ser Phe Asp Ile Leu
865 870 875 880

Asn Ile His Tyr Pro Arg Pro Asn Pro Ser Ile Ala Asn Leu His Val
885 890 895

Glu Gly Glu Ile Ile Ser Ser Pro Asn Asn Ile Cys Lys Leu Asn Ala
900 905 910

Thr Leu Thr Glu Lys Lys Glu Pro Ile Pro Ile Ser Ile Gln Ala Thr
915 920 925

Leu Pro Phe Glu Phe Thr Glu Asn Asn Ile Pro Met Leu Ser Lys Met
930 935 940

Arg Pro Phe Ser Ala His Ile Lys Trp Thr Gly Ile Leu Asp Thr Leu
945 950 955 960

Trp Lys Leu Ile Pro Leu Thr Asp Tyr Ile Met Ala Gly Asn Gly Ser
965 970 975

Leu Asp Ala Ser Leu Ser Gly Thr Leu Asp Ser Pro Thr Tyr Ala Ile
980 985 990

Ile Thr Thr Leu Ser Asn Ala Asn Phe Gln Asp Leu Ser Leu Gly Leu
995 1000 1005

Tyr Leu Glu Asn Ile Asn Ala Lys Leu Gln Val Phe Ser Asn Arg Ile

1010 1015 1020

Ser His Ile Gln Ala Thr Ala Ser Asp Gly Lys Gln Gly Ser Ile Gln
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Leu Ile Gly Asn Ile Gly Ser Ser Lys Glu His Phe Pro Leu Ser Ile
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Asn Gly Ser Phe Thr Asn Leu Ala Pro Leu Gln Arg Lys Asp Leu Ser
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Glu Val Lys Gly Asp Ile Val Ile Asn Gln Gly Glu Phe Gln Leu Thr
1090 1095 1100

Glu Gly Leu Thr Ser Asn Ile Pro Thr Leu Asn Val Val Asp Ser Thr
1105 1110 1115 1120

Gln Gln Gln Asn Thr Lys Thr Lys Lys Ala Thr Tyr Gln Gln Pro Thr
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Thr Ser Pro Val Ile Thr Gly Ala Leu Thr Ser Ile Arg Gly Asn Phe
1170 1175 1180

Asn Leu Leu Gly Lys Gln Phe Ser Leu Ala Lys Ser Thr Ile Ser Phe
1185 1190 1195 1200

Ser Gly Ser Val Pro Pro Asn Pro Leu Leu Asn Ile Ser Leu Thr Tyr
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1250 1255 1260

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1315 1320 1325

Lys Tyr Ile Thr Asp Thr Val Tyr Val Gly Val Glu Gln Ser Tyr Leu
1330 1335 1340

Asp Ser Asn Asp Thr Gly Ala Arg Ile Ser Val Glu Leu Ala Pro Asn
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WE CLAIM:

1. An isolated or recombinant immunogenic polypeptide which comprises, mimics or cross-reacts with a B-cell or T-cell epitope of a *Lawsonia spp.* polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides.

2. The isolated or recombinant immunogenic polypeptide of claim 1 capable of eliciting the production of antibodies against *Lawsonia spp.* when administered to an avian or porcine animal.

3. The isolated or recombinant immunogenic polypeptide of claim 1 capable of conferring a protective immune response against *Lawsonia spp.* when administered to an avian or porcine animal.

4. The isolated or recombinant immunogenic polypeptide of claim 2 wherein the *Lawsonia spp.* is *L. intracellularis.*

5. The isolated or recombinant immunogenic polypeptide of claim 3 wherein the *Lawsonia spp.* is *L. intracellularis.*

6. An isolated or recombinant polypeptide selected from the group consisting of:
(i) a polypeptide of *Lawsonia spp.* which comprises an amino acid sequence which has at least about 60% sequence identity overall to an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, and 18;
(ii) a polypeptide of *Lawsonia spp.* which comprises an amino acid sequence which has at least about 60% sequence identity overall to an amino acid sequence encoded by *Lawsonia intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480

(plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM);

(iii) a polypeptide which comprises at least about 5 contiguous amino acids of an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, and 18;

(iv) a polypeptide which comprises at least about 5 contiguous amino acids of an amino acid sequence encoded by *Lawsonia intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM); and

(v) a polypeptide which comprises an amino acid sequence encoded by a nucleotide sequence of *Lawsonia spp.* having at least about 60% sequence identity overall to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17;

(vi) a polypeptide which comprises an amino acid sequence encoded by a nucleotide sequence of *Lawsonia spp.* having at least about 60% sequence identity overall to the nucleotide sequence of *Lawsonia intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM);

(vii) a polypeptide encoded by at least about 15 contiguous nucleotides of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17;

(vii) a polypeptide encoded by at least about 15 contiguous nucleotides of a nucleotide sequence of *Lawsonia intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478

(plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM); and

(viii) a homologue, analogue or derivative of any one of (i) to (vii) which mimics a B-cell or T-cell epitope of *Lawsonia spp.*

7. The isolated or recombinant polypeptide of claim 6 capable of eliciting the production of antibodies against *Lawsonia spp.* in a porcine or avian animal.

8. The isolated or recombinant polypeptide of claim 7 capable of conferring a protective immune response against *Lawsonia spp.* in a porcine or avian animal.

9. The isolated or recombinant polypeptide of claim 8, capable of inducing humoral immunity against *Lawsonia spp.* in a porcine or avian animal.

10. The isolated or recombinant polypeptide of claim 9, capable of inducing humoral immunity against *Lawsonia spp.* in a porcine animal.

11. The isolated or recombinant polypeptide of claim 8 wherein the *Lawsonia spp.* is *Lawsonia intracellularis.*

12. The isolated or recombinant polypeptide of claim 10 wherein the *Lawsonia spp.* is *Lawsonia intracellularis.*

13. The isolated or recombinant polypeptide of claim 6 comprising an amino acid sequence selected from the group consisting of:

(i) an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, and 18; and

(ii) an amino acid sequence encoded by *Lawsonia intracellularis* DNA contained within a deposited plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid

pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM).

14. The isolated or recombinant polypeptide of claim 13 capable of eliciting the production of antibodies against *Lawsonia intracellularis* when administered to an avian or porcine animal.

15. The isolated or recombinant polypeptide of claim 13 or 14 capable of inducing a protective immune response against *Lawsonia intracellularis* in a porcine or avian animal.

16. The isolated or recombinant polypeptide of claim 15 capable of inducing a protective immune response against *Lawsonia intracellularis* in a porcine animal.

17. A vaccine composition for the prophylaxis or treatment of infection of an animal by *Lawsonia spp.*, said vaccine composition comprising an immunogenic component which comprises the isolated or recombinant immunogenic polypeptide according to claim 1 in combination with one or more carriers, diluents or adjuvants suitable for veterinary or pharmaceutical use.

18. The vaccine composition according to claim 17 wherein the *Lawsonia spp.* is *Lawsonia intracellularis*.

19. The vaccine composition according to claim 17 wherein the immunogenic component comprises an isolated or recombinant polypeptide having an amino acid sequence selected from the group consisting of:

(i) an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, and 18; and

(ii) an amino acid sequence encoded by *Lawsonia intracellularis* DNA contained within a deposited plasmid selected from the group consisting of AGAL

Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM).

20. The vaccine composition of claim 17, wherein the immunogenic component is a recombinant polypeptide expressed in a cell that has been transfected with a vector comprising a nucleotide sequence selected from the group consisting of:

(i) a protein-encoding nucleotide sequence having at least about 60% sequence identity overall to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17;

(ii) a protein-encoding nucleotide sequence having at least about 60% sequence identity overall to the protein-encoding sequence of *Lawsonia intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM);

(iii) a protein-encoding nucleotide sequence which comprises at least about 15 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17;

(iv) a protein-encoding nucleotide sequence which comprises at least about 15 contiguous nucleotides of the protein-encoding sequence of *Lawsonia intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM);

(v) a protein-encoding nucleotide sequence which hybridizes under at least low stringency conditions to the complement of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17;

(vi) a protein-encoding nucleotide sequence which hybridizes under at least low stringency conditions to the non-coding strand of *Lawsonia intracellularis* DNA contained within a p plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM); and

(vii) a homologue, analogue or derivative of any one of (i) to (vi) which encodes a polypeptide which mimics a B-cell or T-cell epitope of *Lawsonia spp.*

21. A combination vaccine composition for the prophylaxis or treatment of infection of an animal by *Lawsonia spp.*, said vaccine composition comprising:

(i) a first immunogenic component which comprises the isolated or recombinant polypeptide having according to claim 1;

(ii) a second immunogenic component different from said first immunogenic component and comprising a polypeptide selected from the group consisting of the *Lawsonia intracellularis* FlgE, hemolysin, OmpH, SodC, flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides; and

(iii) one or more carriers, diluents or adjuvants suitable for veterinary or pharmaceutical use.

22. A vaccine vector that comprises, in an expressible form, an isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

(i) a protein-encoding nucleotide sequence having at least about 60% sequence identity overall to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17;

(ii) a protein-encoding nucleotide sequence having at least about 60% sequence identity overall to the protein-encoding sequence of *Lawsonia intracellularis* DNA contained within a p plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid

pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM);

(iii) a protein-encoding nucleotide sequence which comprises at least about 15 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17;

(iv) a protein-encoding nucleotide sequence which comprises at least about 15 contiguous nucleotides of the protein-encoding sequence of *Lawsonia intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM);

(v) a protein-encoding nucleotide sequence which hybridizes under at least low stringency conditions to the complement of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17;

(vi) a protein-encoding nucleotide sequence which hybridizes under at least low stringency conditions to the non-coding strand of *Lawsonia intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM); and

(vii) a homologue, analogue or derivative of any one of (i) to (vi) which encodes a polypeptide which mimics a B-cell or T-cell epitope of *Lawsonia spp.*

wherein said vaccine vector expresses the polypeptide encoded by said nucleotide sequence at a level sufficient to confer immunity against *Lawsonia spp.* when administered to a porcine or avian animal.

23. The vaccine vector of claim 22 wherein the immunogenic polypeptide is expressed by a process comprising:

(i) placing an isolated nucleic acid molecule in an expressible format, said

nucleic acid molecule comprising the coding region of a gene selected from the group consisting of *flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM*, and *ytfN* genes, or a protein-encoding homologue, analogue or derivative thereof;

(ii) introducing the isolated nucleic acid molecule of (i) in an expressible format into a suitable vaccine vector; and

(iii) incubating or growing the vaccine vector for a time and under conditions sufficient for expression of the immunogenic component encoded by said nucleic acid molecule to occur.

24. The vaccine vector of claim 22 wherein the *Lawsonia spp.* is *L. intracellularis.*

25. An isolated polyclonal antibody or a monoclonal antibody molecule that binds specifically to a *Lawsonia spp.* polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides, or a homologue, analogue or derivative of any one or more of said polypeptides.

26. The isolated polyclonal antibody or a monoclonal antibody molecule of claim 25 wherein the polypeptide or derivative thereof comprises an amino acid sequence selected from the group consisting of:

(i) an amino acid sequence of *Lawsonia sp.* which has at least about 60% sequence identity overall to a sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, and 18;

(ii) an amino acid sequence of *Lawsonia sp.* which has at least about 60% sequence identity overall to a sequence encoded by *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM);

(iii) an amino acid sequence which comprises at least about 5 contiguous amino acids of a sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6,

8, 10, 12, 14, 16, and 18;

(iv) an amino acid sequence which comprises at least about 5 contiguous amino acids of a sequence encoded by *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM);

(v) an amino acid sequence which is encoded by a nucleotide sequence having at least about 60% sequence identity overall to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17;

(vi) an amino acid sequence which is encoded by a nucleotide sequence having at least about 60% sequence identity overall to the nucleotide sequence of *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM);

(vii) an amino acid sequence which is encoded by at least about 15 contiguous nucleotides of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17;

(viii) an amino acid sequence which is encoded by at least about 15 contiguous nucleotides of a nucleotide sequence of *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM); and

(ix) a homologue, analogue or derivative of any one of (i) to (viii) which mimics a B-cell or T-cell epitope of *Lawsonia spp.*

27. A method of diagnosing infection of a porcine or avian animal by *Lawsonia intracellularis* or a microorganism that is immunologically cross-reactive thereto, said method comprising the steps of contacting a biological sample derived from said animal with the antibody molecule of claim 25 for a time and under conditions sufficient for an antigen:antibody complex to form, and then detecting said complex formation.

28. The method of claim 27 wherein the biological sample comprises whole serum, lymph nodes, ileum, caecum, small intestine, large intestine, faeces or a rectal swab derived from a porcine animal.

29. A method of identifying whether or not a porcine or avian animal has suffered from a past infection, or is currently infected, with *Lawsonia intracellularis* or a microorganism that is immunologically cross-reactive thereto, said method comprising contacting blood or serum derived from said animal with the immunogenic polypeptide of claim 1 for a time and under conditions sufficient for an antigen:antibody complex to form and then detecting said complex formation.

30. An isolated nucleic acid molecule which consists of a nucleotide sequence encoding a *Lawsonia spp.* polypeptide selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN.

31. The isolated nucleic acid molecule according to claim 30 comprising a sequence of nucleotides selected from the group consisting of:

(i) a nucleotide sequence having at least about 60% sequence identity overall to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17;

(ii) a nucleotide sequence having at least about 60% sequence identity overall to *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR);

NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM);

(iii) a nucleotide sequence which comprises at least about 15 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17;

(iv) a nucleotide sequence which comprises at least about 15 contiguous nucleotides of *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM);

(v) a nucleotide sequence which hybridizes under at least low stringency conditions to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17 or a complementary nucleotide sequence thereto;

(vi) a nucleotide sequence which hybridizes under at least low stringency conditions to *L. intracellularis* DNA contained within a plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM); and

(vii) a homologue, analogue or derivative of any one of (i) to (vi) which encodes a polypeptide which mimics a B-cell or T-cell epitope of *Lawsonia spp.*

32. The isolated nucleic acid molecule of claim 31 comprising a nucleotide sequence selected from the group consisting of:

(i) a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1,3, 5, 7, 9, 11, 13, 15, and 17;

(ii) a nucleotide sequence of the *L. intracellularis* DNA contained within a deposited plasmid selected from the group consisting of AGAL Accession Nos:

NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM); and

(iii) a nucleotide sequence that encodes the same polypeptide as a nucleotide sequence of (i) or (ii), wherein said polypeptide is selected from the group consisting of flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM, and ytfN polypeptides; and

(iv) a nucleotide sequence that is complementary to a nucleotide sequence of (i) or (ii) or (iii).

33. The isolated nucleic acid molecule of claim 32 consisting of the protein-encoding region of (i) or (ii).

34. A method of detecting *Lawsonia intracellularis* or related microorganism in a biological sample derived from a porcine or avian animal subject, said method comprising the steps of hybridising one or more probes or primers to said sample and then detecting said hybridisation using a detection means, wherein said probes or primers are derived from a *Lawsonia spp.* gene selected from the group consisting of *flhB, fliR, ntrC, glnH, motA, motB, tlyC, ytfM*, and *ytfN* genes.

35. The method of claim 34 wherein the biological sample comprises whole serum, lymph nodes, ileum, caecum, small intestine, large intestine, faeces or a rectal swab derived from a porcine animal.

36. The method of claim 34 wherein the detection means comprises any nucleic acid based hybridisation or amplification reaction.

37. A probe or primer comprising a nucleotide sequence selected from the group consisting of:

(i) any one of SEQ ID NOs: 19 to 46; and

(ii) a complementary nucleotide sequence to (i).

38. A plasmid selected from the group consisting of AGAL Accession Nos: NM00/16476 (plasmid pGTE#1 glnH); NM00/16477 (plasmid pGTE#2 flhB); NM00/16478 (plasmid pGTE#3 fliR); NM00/16479 (plasmid pGTE#4 motA/B); NM00/16480 (plasmid pGTE#5 tlyC); NM00/16481 (plasmid pGTE#6 ntrC); and NM00/16482 (plasmid pGTE#7 ytfM).

39. A recombinant vector capable of replication in a host cell, wherein said vector comprises the isolated nucleic acid of claim 30.

40. A recombinant vector capable of replication in a host cell, wherein said vector comprises the isolated nucleic acid of claim 31.

41. A recombinant vector capable of replication in a host cell, wherein said vector comprises the isolated nucleic acid of claim 32.

42. A recombinant vector capable of replication in a host cell, wherein said vector comprises the isolated nucleic acid of claim 33.

43. A host cell comprising the recombinant vector of claim 39.

44. The host cell of claim 43 wherein said host cell is a bacterium.

45. A host cell comprising the recombinant vector of claim 40.

46. The host cell of claim 45 wherein said host cell is a bacterium.

47. A host cell comprising the recombinant vector of claim 41.

48. The host cell of claim 47 wherein said host cell is a bacterium.

49. A host cell comprising the recombinant vector of claim 42.

50. The host cell of claim 49 wherein said host cell is a bacterium.

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